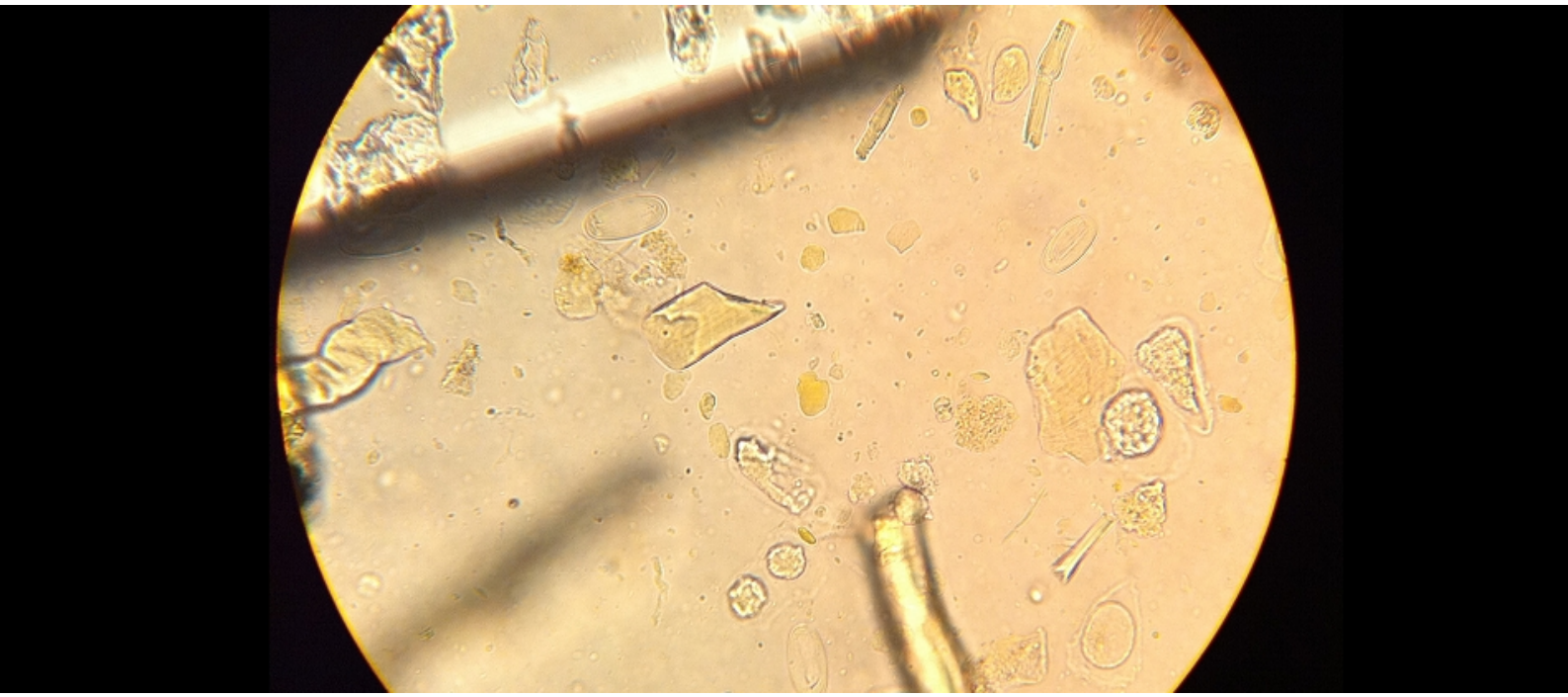
Spirocerca lupi infections in dogs on the island of Curaçao

A Survey



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Abstract

In August and September 2011, 157 dogs were examined for the presence of a *Spirocerca lupi* infection. A fecal sample was examined with the use of the standard Centrifugation Sedimentation Flotation (CSF)-method using a sugar solution. The slide was then examined systematically under the microscope. Also information on the dog, such as age, breed, gender, risk behaviours, treatments and preventative measures and clinical signs was gathered through a questionnaire.

In this investigation a *S. lupi* prevalence was found of 10.2%. No association was found when looking at sex, reproductive state, being a shelter or domestic dog or geographical location of the dogs. However a significant association was found between the age group “1-3 years” and having an infection. A predisposition for large breeds might exist, but was not significantly proven in this investigation.

No direct association was found when looking at the risk behaviours: eating raw meat, coprophagy and hunting. This does not mean that these behaviours are not important routes of infection. Also no effect on the infection rate was seen in the primary use of dogs, how and where they were kept and the use of a kennel. The use of insecticides did not prove to be effective in diminishing the risk. Also no association was seen between having an infection and the dewormingschedule or the use of Heartgard. The use of Ivomec however did seem to have some effect on the infection rate.

Most of the *Spirocerca lupi* infections were subclinical. The signs observed in clinical cases were anorexia, vomiting, diarrhea and weight loss.

1. Introduction

Spirocercosis in dogs is caused by the nematode *Spirocerca lupi*. Although the parasite is found all over the world, it is mostly seen in tropical areas [van der Merwe et al., 2008]. This parasite has an indirect life cycle, with coprophagous beetles as the intermediate host [Taylor et al., 2007]. Different species of carnivores can be the final host, although dogs are the most affected. Other examples are the wolf, the lynx and the cat. They can acquire an infection by consuming the intermediate host or one of the many paratenic hosts. After ingestion, the parasite excysts in the stomach and penetrates the gastric wall. It migrates through the a. gastrica, a. epiploica and the a. coeliaca to reach the aorta. In the aorta the worm stays for up to 3 months and develops into a more mature state [Bailey, 1972]. The parasite ends up in the caudal esophagus where it forms a nodule. Female worms excrete their eggs into the esophageal lumen. These eggs (containing a L1 larva) are then excreted into the environment with the dog's feces and vomit [Chhabra and Singh, 1972]. The cycle is complete after the coprophagous beetle ingests the excreted eggs. Development of L1 to L3 takes place in these beetles.

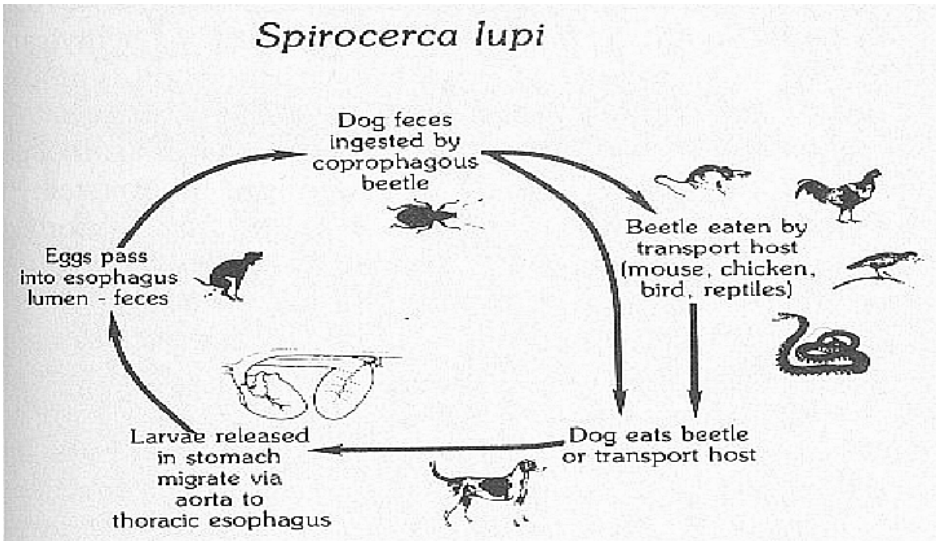


Figure 1: *S. lupi* life cycle
Source: Fox et al., 1988

Larval migration and the nodules which are formed are the cause of many of the clinical signs in the dog [van der Merwe et al., 2008]. For the large part these consist of gastrointestinal and respiratory problems. Vomiting, regurgitation and dysfagia are often followed by anorexia, weakness and weight loss. Eventually the dog reaches a lethargic and emaciated state. Blood loss is caused by larval migration and the formation of nodules in the esophagus. This results in melena and anemia. Dogs can suddenly die in case of an aneurism of the aorta. The general inflammation reaction causes a fever (rectal temperature > 39.5°C). If the worm deviates from the normal migratory route, aspecific clinical signs can develop.

The diagnosis can sometimes be challenging, but with the use of fecal examination, thoracic radiography or endoscopy, it can be established. The most effective therapy in uncomplicated cases is treatment with the macrocyclic lactones doramectin or ivermectin [van der Merwe et al., 2008]. When treating a collie or other dog breed, it is important to be careful using this group of anthelminthics, because of the genetic sensitivity for an intoxication [Wijnands-Kleukers et al., 1996]. The same is the case when treating a pregnant bitch, as the blood placenta barrier is sometimes permeable for these medicines and the blood brain barrier of the foetuses is not fully functional yet. The treatment leads to a decrease in egg shedding, kills the adult parasites and makes the esophageal nodules go into regression. If the nodules underwent neoplastic transformation, the treatment could consist of esophageal surgery combined with the chemotherapeutic agent doxorubicin [Ranen et al., 2004]. This therapy is not without risks, as complications often occur in esophageal surgery. Prevention is achieved by reducing the infection pressure in the environment by treating the infected dogs and disposing of their feces [van der Merwe et al., 2008]. Applying pest control and monitoring the uptake of possible infective material by the dog are other ways of decreasing the chance of an infection.

2. Aim of the study

Up to the 10th of October 2010 Curaçao was part of the Netherlands Antilles [Central Intelligence Agency, 2011]. Now it is a constituant country of the Kingdom of the Netherlands. It is located in the southern Caribbean sea, just of the coast of the South American country Venezuela. The island has a tropical climate, with an average high temperature of 31.2 degrees Celcius. With a population of more than 142.000 inhabitants and a surplus of dogs, the demand for veterinary care is evident.

Spirocercosis in dogs is an unknown condition among dog owners on the island of Curaçao, however it is a condition which is seen regularly and can have serious consequences for a dog's health. The only study on the prevalence of *S. lupi* in dogs in Curaçao was done in 1986 [Saleh et al., 1988]. In this study 133 dogs were examined for evidence of gastrointestinal parasites by fecal examination. Dogs were classified as either being a pet (n = 74) or feral (n = 59). They found that a Spirocerca infection was present in 5.4% of the pet dog population and in 3.4% of the feral population. They used the formalin-ethyl acetate sedimentation technique, instead of the fecal flotation or dilution methods. The latter are considered to be more sensitive methods in diagnosing spirocercosis. Therefore these results might have underestimated the real prevalence. More research needs to be done on the prevalence and mode of infection.

The main goal of this study was to determine the prevalence and the distribution of *S. lupi* infections in dogs on Curaçao. Also important was to determine which clinical signs are associated with spirocercosis on the island and what risk behaviours contribute to acquiring an infection. With this information practicing veterinarians are better able to assess if a dog is infected with the parasite and can start or adjust therapy accordingly. Another goal of the study was to determine the percentage of dogs infected with the parasite which developed an esophageal sarcoma. Also a study was done on shelter dogs to determine the prevalence of a *Spirocerca lupi* infection.

3. Materials & methods

The investigation took place in a period of 2 months, August and September, during 2011. In total three veterinary practices and 1 dog walking service/vacation park were involved. Shelter dogs were also included.

In total 157 dogs were sampled, either rectally or by a spontaneous discharge. Also information on the dog, such as age, breed, gender, risk behaviours, treatments and preventative measures and clinical signs was gathered through a questionnaire (appendix 1).

The fecal sample was kept refrigerated until examination in the laboratory. The laboratory protocol consisted of the standard Centrifugation Sedimentation Flotation (CSF)-method (appendix 2) using a sugar solution. The sugar solution had a specific gravity of 1.3. Because the examinator had trouble with this solution, a modified sugar solution was made which was calibrated by using a fecal sample which was tested positive for *S. lupi* eggs using the first solution.

The slide was examined systematically under the microscope by using the 10 (ocular) x 10 (objective) magnification. All parasite eggs found were registered in an excell document. The cooperating practices received an update of the results of the examination every 2 weeks, after which they had the opportunity to notify the dog owners.

Examining the shelter dog population, the same laboratory protocol was used. Instead of the questionnaire, registered information on the dog was printed out. This included descriptives (e.g. age etc.) on the dog and any treatments the dog may have had during his time at the shelter.

Performing necropsy on euthanised animals was also part of the investigation. The goal was to establish if and how many dogs suffered from a esophageal sarcoma and of course to see the pathological picture associated with a *Spirocerca lupi* infection in real life. Necropsy was performed on only 4 dogs. The dogs were euthanised by injecting Euthasate ® intravenously. To verify that the dog had died, the heart was listened to. The dog was strapped on a table on his back, and an incision was made from the cranial sternum to the abdomen. The skin was prepared loose and the ribs were cut to reach the thoracic cavity. In the thorax the aorta and the caudal esophagous were examined for signs of a current or past infection. Also the heart was cut open to see if the dog had a heartworm infection. In the abdomen the stomach was examined for signs of larval migration. Note that this is not a protocol used by Utrecht University, it was used only in this study to determine whether there was a *Spirocerca lupi* infection.

4. Results

4.1 *S. lupi* infections

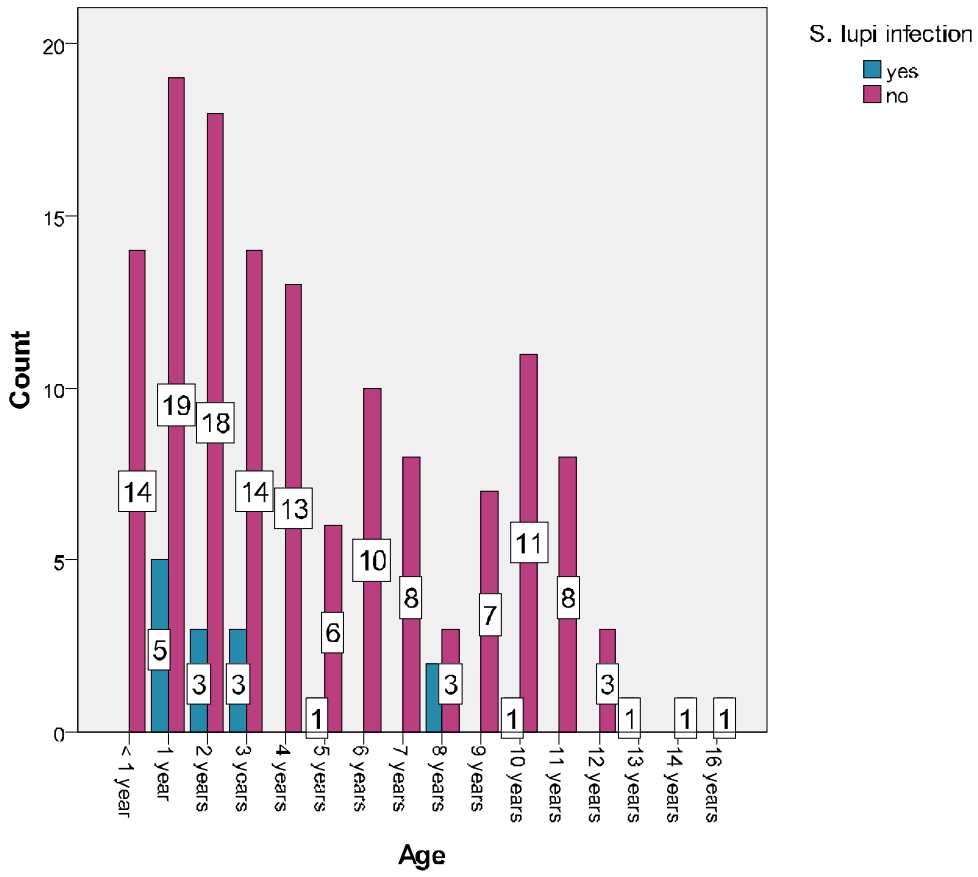
In total 16/157 dogs (10.2%) with CI-95% = [.05; .15], were found infected with *S. lupi* with the use of the CSF method. The true prevalence is expected to be higher, because a fraction of infected dogs do not shed eggs [Harrus et al., 1996]. 101 samples were collected from a spontaneous deposit by the dog and 56 from a fecal loop. 10 out of the 101 “spontaneous dogs” (9.9%) had an infection, while 6 out of 50 “loop dogs” (10.7%) were found infected.

4.1.1 Descriptives

Age

The age of the participating dogs ranged from 6 months to 17 years old (graph 1). The mean age was 5.67 years. Of the 16 infected dogs, 11 (69%) were found in the age group 1 to 3 years old, while this age group makes out only 51/157 (32%) of the total dog population. The mean age of the infected dogs was 3 years old. The mode age was 1 year, for both *S. lupi* infections and in general.
A significant association was present between age, more specifically the age group 1 to 3 years, and having a *S. lupi* infection (χ^2 (1)= 5.789, p < 0.05). This supports the fact that based on the odds ratio dogs in the age group 1 to 3 were 3.67 times more likely to be infected.

Graph 1 – Age and *S. lupi* infections



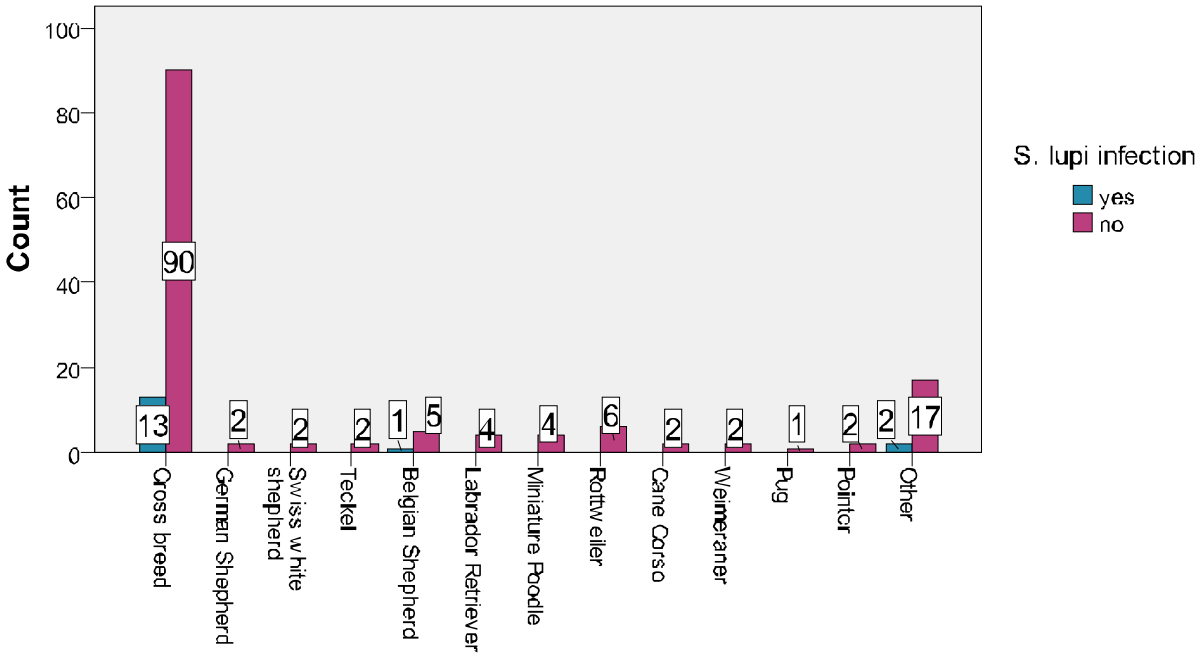
Breed

Most of the dogs taking part in this investigation were of a cross breed (103/157) (graph 2). The rottweiler (6) and belgian shepard dog (5) were the most represented pure breed dogs in this study. In total 30 different breeds were recorded in the study. Breeds with only one dog in the sample, were collected in the “other” category.

Of the *S. lupi* infected dogs 13/16 (81.3%) were found in the cross breed category, while this category makes out 66.5% of the total sample. The 3 other infected dogs were of a pure breed: belgian shepherd, dutch shepherd and rhodesian ridgeback.

When using the Chi-square test to assess the association between a cross breed and having a *S. lupi* infection, a non-significant result was found (χ^2 (1)= 1.753, p > 0.05).

Graph 2 – Breed and *S. lupi* infections



Sex

This study included 60 male and 97 female dogs. Of the female sample 12/97 (12.4%) was infected, while only 4/60 males (6.7%) had the infection. However, a non-significant association was present between sex and having a *S. lupi* infection (χ^2 (1)= 1.318, p > 0.05).

Reproductive state

In total 62.5% of the sampled dogs was neutered. The distribution among the sexes was unequal (females 75.6% and males 39.1%).

Of the infected dogs 10/12 (83.3%) were neutered, opposed to 70/116 (60.3%) of the not infected dogs. Among the infected males 2/3 were neutered, while 16/43 (37.2%) of the non infected males was neutered. In the females sample these values were 8/9 (88.9%) and 54/73 (74.0%) for infected and non infected respectively.

Based on the percentages, there seems to be an association in the male category between reproductive state and being infected. When using the Chi-square test, this seems to be non-significant (χ^2 (1)= 1.022, p > 0.05). Because of the small number of infected dogs, the conditions for using the Chi-square test weren't met. Therefore this result might be less reliable.

Domestic or shelter dog

Among the population shelter dogs, 26 dogs were sampled. Twelve out of the 26 dogs were male, and 14 dogs were female. The sampled domestic dogs consisted of 131 dogs.

13 out of 131 domestic dogs (9.9%) had an infection, while 3 out of 26 shelter dogs (11.5%) were infected.

Geographical distribution

Most of the domestic dogs in this investigation lived in the eastern part of the island (Map 1), more specifically the area around the capital Willemstad. Geographically the infected dogs seem to be evenly dispersed over the sample.

Map 1 – Geographical distribution of domestic dogs

4.1.2 Risk Behaviour

34 dogs (27.0%) were given raw animal products by their owner. In this group, 3 dogs (8.8%) were found infected. In the “not given raw animal products” group, 10 dogs (10.9%) had a *S. lupi* infection. No association is expected based on these percentages.

According to the owner, 23 dogs have ever eaten dog poop. Among these dogs, 3 (13.0%) were infected. In the not “eating dog feces” group, 9.8% had an infection. These percentages do not lie far from each other, therefore no association is expected.

107 dogs were considered a hunter by their owners, while only 20 dogs never seemed to hunt. Among the hunters, 11 dogs (10.3%) were infected. In the not hunting group, 2 dogs (10.0%) had a *S. lupi* infection. Again, no association is expected based on these numbers.

In the case of 106 dogs, the owner said to have found dead animals in the yard sometimes. In this group, 11 (10.4%) dogs were infected. In the other group “never finding dead animals in the yard”, the percentage of infected animals was 9.5%. Also in this category, no association is supported.

4.1.3 Management

Primary use, kept where and kept how

85 dogs (65.9%) were used primarily as a companion animal, 8 owners (6.2%) used their dog only as a guard dog, and 36 owners (27.9%) considered their dog both a companion animal and a guard dog. The categories are ranked from high to low according to the proportions of infected dogs: guard dog

(14.3%), companion animal (11.8%) and both (9.1%). Based on these percentages, there seems to be some influence from the primary use of dogs on having an infection. When using the Chi-square test, no significant association was present (χ^2 (2)= 0.197, p > 0.05).

15 dogs (11.9%) are kept only inside, 57 (45.2%) dogs are kept only outside, and the remaining 54 (42.9%) dogs are kept both inside and outside (table 1). The percentages of infected dogs which are kept only inside and only outside were 13.3% (2/15) and 15.8% (9/57) respectively. Among the dogs kept both inside and outside, 3.7% (2/54) was infected. The percentage of infected dogs kept both inside and outside, appears to differ from the other categories. However this result is based on only 2 infected dogs.

6/7 guard dogs (85.7%) are kept only outside and only 1 is kept both inside and outside. Dogs used for both companion and guarding are also mostly kept only outside (54.3%), but also in and outside (34.3%). In only 11.4% of the cases these dogs are kept only inside. Dogs considered only a companion animal are kept inside the most (13%). But in 48% of the cases these dogs are kept both inside and outside and in 38% of the cases they are kept only outside. The infected guard dog (1) and the 3 dogs used for both guarding and companion were all kept outside, while the infected companion animals (9 dogs) were kept in all sorts of ways.

All dogs on a chain (5) were kept outside (table 1). Of these dogs 4 were companion animals, while 1 was a guard dog. In this category 1 companion animal was found infected. 94 dogs were kept loose, of which 9 dogs (9.6%) were infected.

Table 1 - Primary use * kept where * kept how * and *S. lupi* infection

S. lupi infection				Primary use			
	Kept how			**Companion animal**	**Guard dog**	**both**	**Total ***
yes	loose	Kept where	Inside	2	0	0	2
			Outside	3	1	2	6
			Part inside, part outside	1	0	0	1
		Total *		**6**	**1**	**2**	**9**
	chain	Kept where	Outside	1			1
		Total *		**1**			**1**
no	loose	Kept where	Inside	8	0	3	11
			Outside	19	1	14	34
			Part inside, part outside	27	1	12	40
		Total *		**54**	**2**	**29**	**85**
	chain	Kept where	Outside	3	1		4
		Total *		**3**	**1**		**4**

* Because of new crosstabulation, totals might have changed compared to what is described previously.

Use of kennel

39 dogs were kept in a kennel, of which 26 were shelter dogs. Shelter dogs were all kept in a kennel. In total 5 dogs (12.8%) who were kept in a kennel and 11 out of 118 dogs (9.3%) who are not kept in a kennel were found infected. The percentages of infected dogs kept in a kennel did not differ much between domestic (15.4%) and shelter dogs (11.5%).

Use of insecticides

No insecticides were used in the case of 78 dogs (61.4%), in the case of 10 dogs (7.9%) the entire yard was sprayed and in 39 cases (30.7%) only part of the yard was treated (graph 5). When looking at the “not using any insecticide” group 6 dogs (7.7%) were found infected. No infected dogs were found in the group which sprayed the entire yard. In the group which sprayed only part of the yard 7 dogs (17.9%) had an infection. However, no significant association could be found (χ^2 (2)= 4.214, p > 0.05).

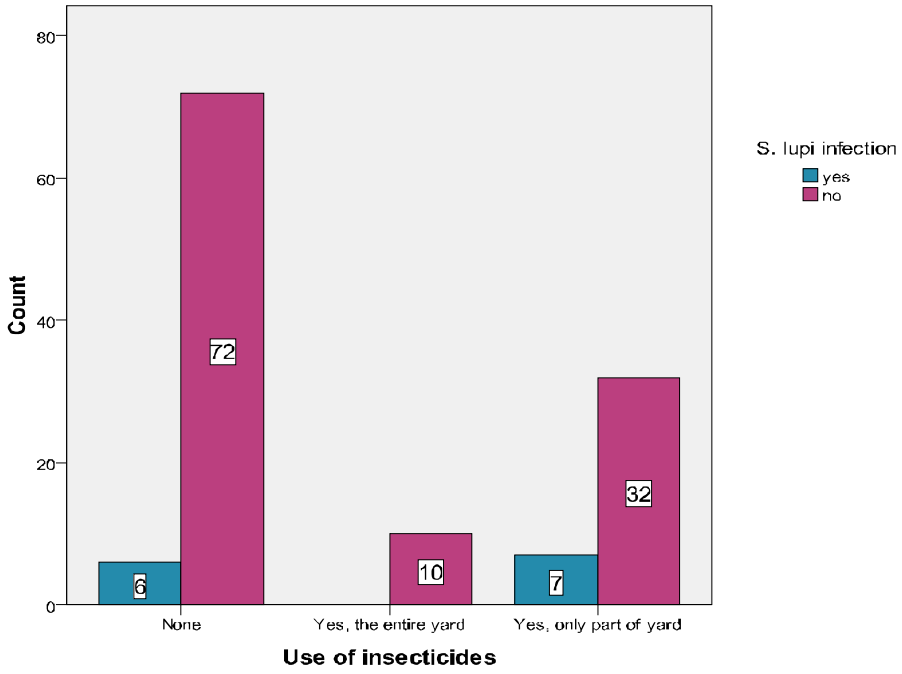
Most people did not know the name of the insecticide they used (Graph 4). Infected dogs were seen in the unknown (6 dogs) and in the Mitaban category (1 dog).

The frequency of use varied from “4 times per year” (13 dogs) to “once every 2 years” (1 dog) (graph 6). There was also a category “irregularly” (12 dogs) and a category “used only once” (3 dogs). No association was found based on the dispersion of the infected dogs in these categories.

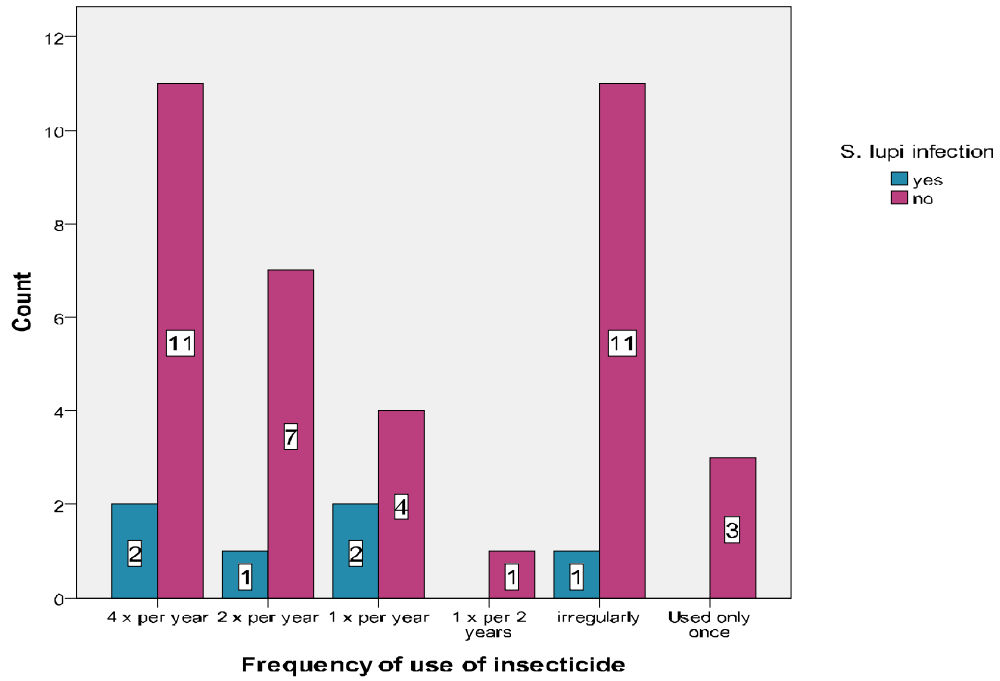
When we are looking at the last time people used the insecticide, we can see that the infected dogs are in the categories “< 3 months” (3 dogs), “3 - 6 months” (2), and “6 months - 1 year” (1) (graph 7).

In total 35 dogs (27.8%) were able to get outside of the yard, of which 4 dogs (11.4%) were infected (table 2). In the group of dogs who were not able to leave the yard 9 dogs (9.9%) had a *S. lupi* infection. We can see that the infection rate of dogs being able to get outside of the yard in the using insecticide group is higher (33.3%) than the infection rate of dogs not being able to leave the yard (11.6%). This difference might seem big, however it is based on only 2 and 5 infected dogs respectively.

Graph 5 – Use of insecticides and *S. lupi* infections



Graph 6 – Frequency of use of insecticide and *S. lupi* infections



Graph 7 – Last use of insecticide and *S. lupi* infections

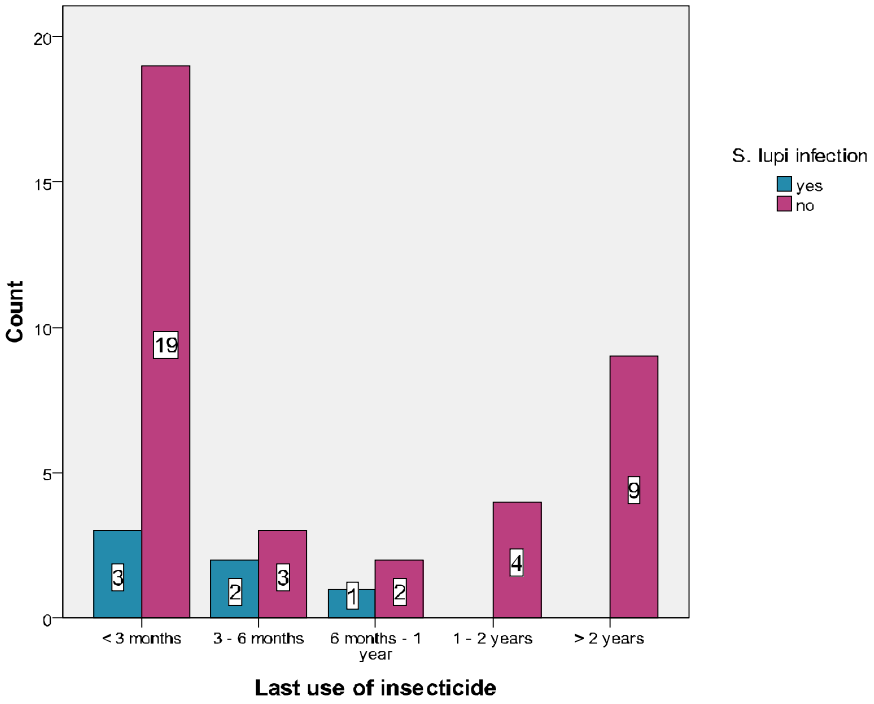


Table 2 - Use of insecticides * *S. lupi* infection * Able to get outside of yard

Able to get outside of yard			*S. lupi* infection		Total
			yes	no	
yes	Use of insecticides	None	2	27	29
		Yes, the entire yard	0	1	1
		Yes, only part of yard	2	3	5
	Total		4	31	35
no	Use of insecticides	None	4	44	48
		Yes, the entire yard	0	9	9
		Yes, only part of yard	5	29	34
	Total		9	82	91

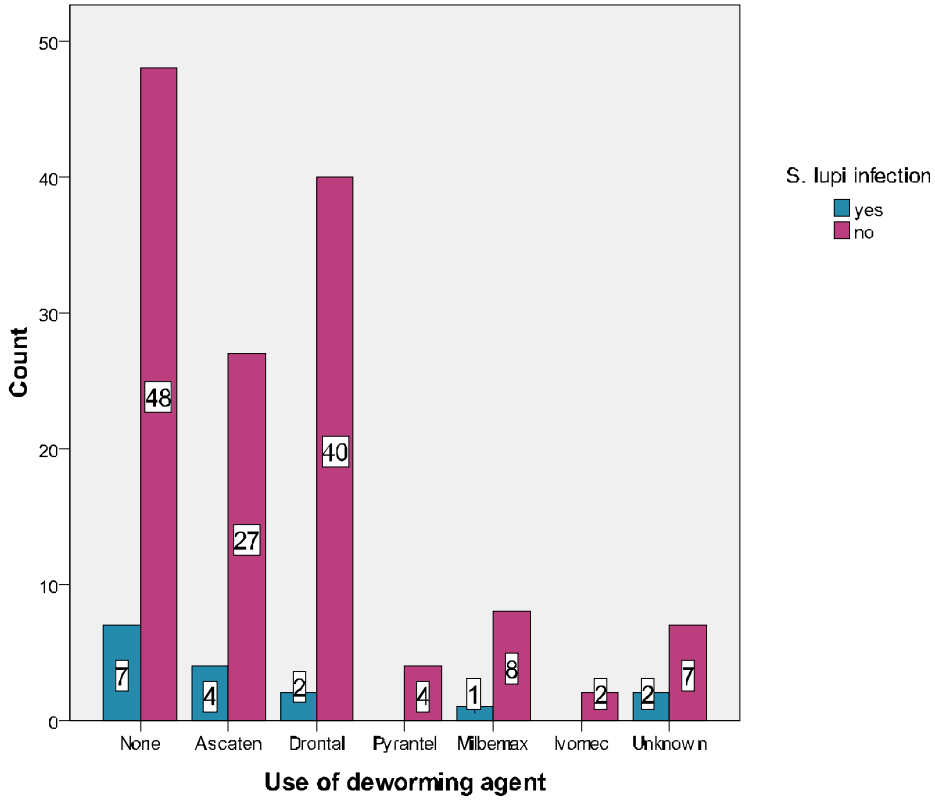
Deworming

55 dogs (36.2%) were not dewormed at all, 42 dogs (27.6%) were dewormed with Drontal® (pyrantelemobonaat, praziquantel and febantel), and in 31 cases (20.4%) Ascaten® (niclosamide and oxybendazol) was used (graph 8). Most *S. lupi* infections (7 dogs) were found in the group which was not dewormed, which makes out 12.7% of this group. The rates of infection in the other groups were 12.9% (Ascaten), 4.8% (Drontal), 11.1% (Milbemax), 22.2% (unknown) and 0% (for both Pyrantel and Ivomec). No association between deworming and an infection could be found (χ^2 (1)= 0.443, $p > 0.05$).

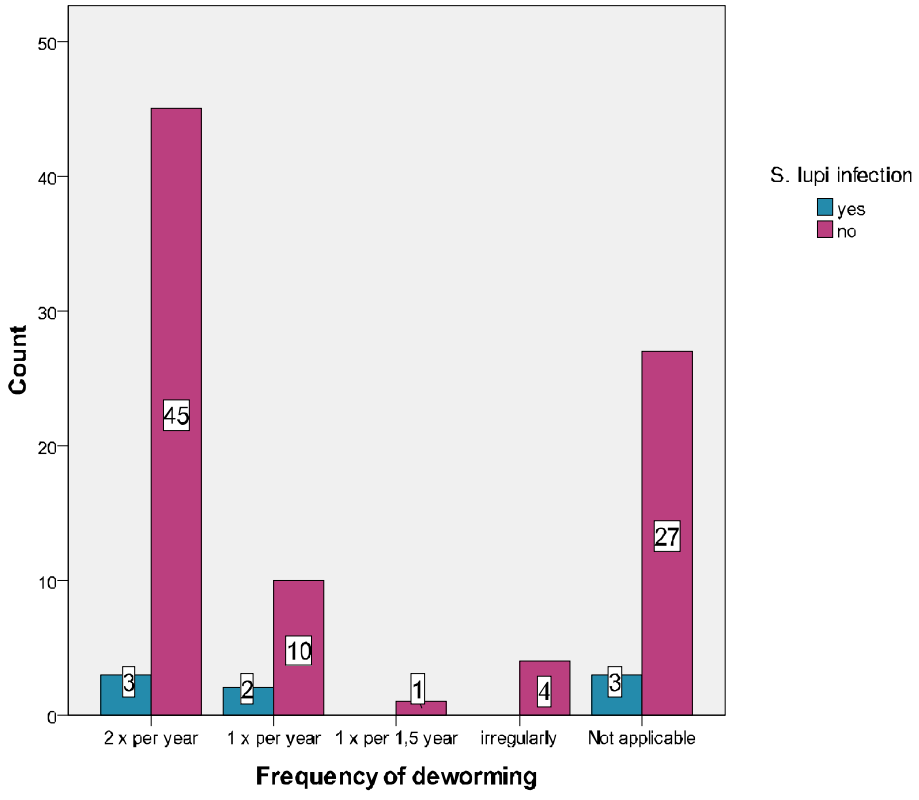
Most dogs (50.5%) were dewormed 2 times a year, as is recommended by veterinarians on the island (graph 9). Consequently, most dogs (86.2%) were last dewormed within a period of 6 months (graph 10).

Heartgard® is a product which is given once a month as a preventive treatment against heartworm disease. The active ingredients are ivermectin and pyrantel. 99 dogs (65.6%) did not use Heartgard at all, while 50 dogs (33.1%) used it once a month (graph 11). Only 2 dogs did not take the Heartgard tablets correctly. In these cases the owner did not administer it frequently enough. 8 infected dogs (8.0%) were found in the not using Heartgard category and 7 infected dogs (14%) were receiving Heartgard correctly.

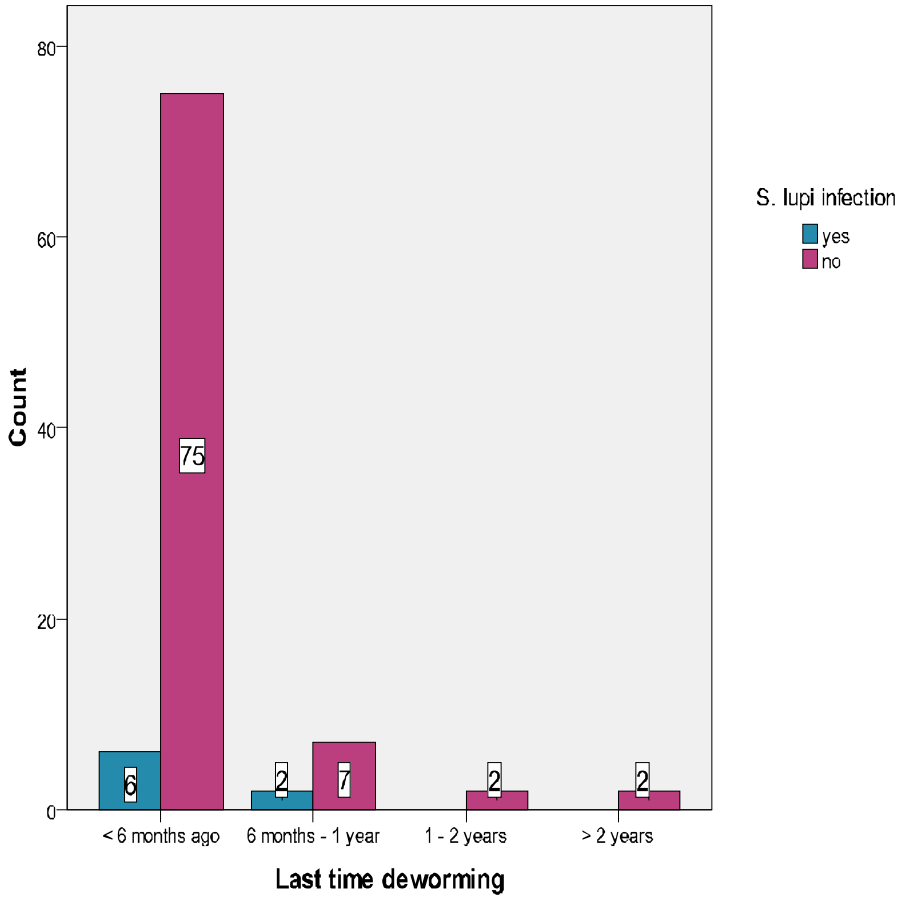
Graph 8 – Deworming and *S. lupi* infections



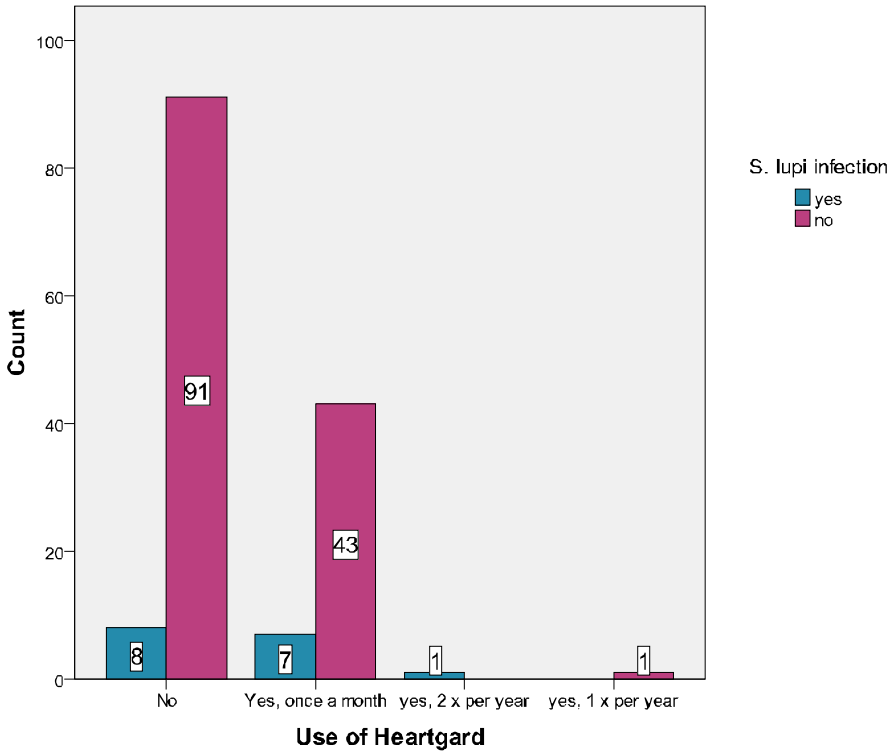
Graph 9 – Frequency of deworming and *S. lupi* infections



Graph 10 – Last time deworming and *S. lupi* infections



Graph 11 – Use of Heartgard® and *S. lupi* infections



Use of agent against flees and ticks

Only 30 out of 150 dogs (20%) did not get any treatment against flees and ticks (graph 12). The most used agent (62 dogs / 41.3%) was Frontline®, with the active ingredients fipronil/methopreen. In 26 cases (17.3%) Ivomec® was used, with the active ingredient ivermectin.

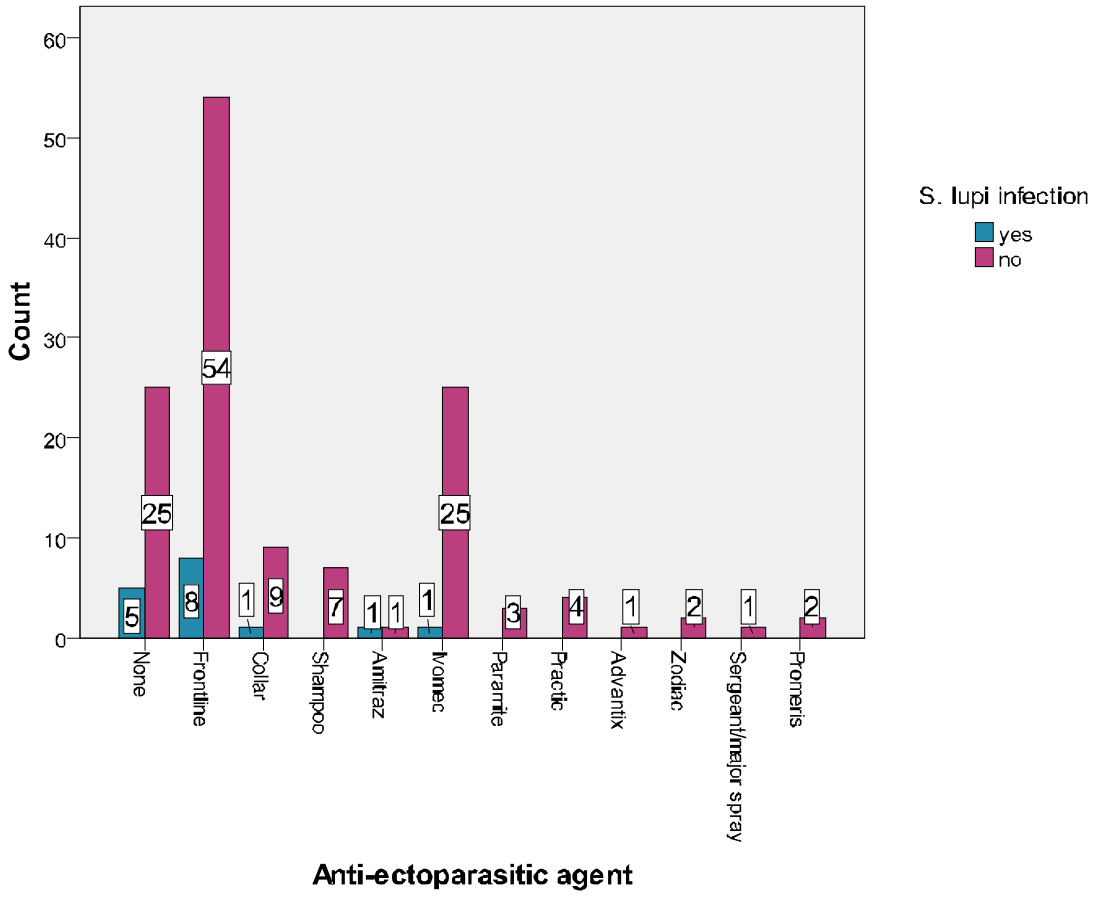
In the not using any treatment group 5 dogs (16.7%) were infected. Of the dogs in the "Frontline" group 8 (12.9%) were infected. Of the dogs using Ivomec only 1 (3.8%) was infected. The other 2 infected dogs were found in the amitraz and using collar group, which in total consisted of 2 and 10 dogs respectively. There seems to be some association between the use of Ivomec and having an infection. However no significant association was found when using the Chi-square test (χ^2 (1)= 1.535, $p > 0.05$). The conditions were not met for using the Chi-square test, therefore this result might not be reliable.

In the case of 52 dogs (46%), the anti-ectoparasitic agent was used once a month. 12.4% of the dogs received this treatment once every 2 months and 8% once every 3 months (graph 13).

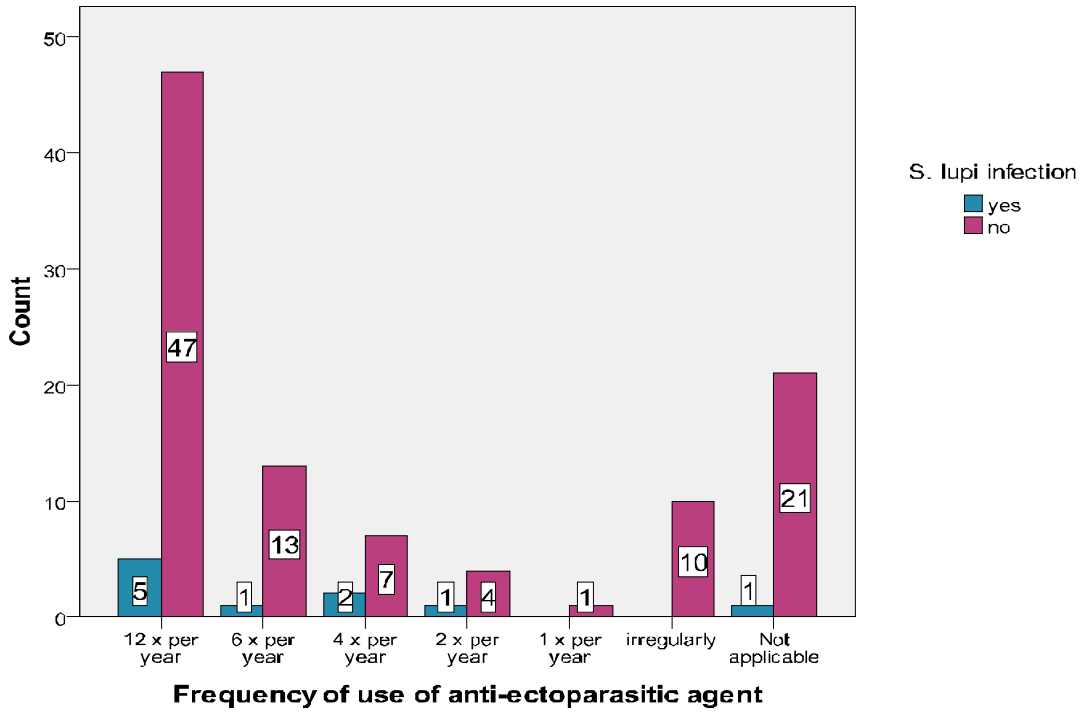
Infected dogs were found in the "12 times a year" group (9.6%), "6 times a year" group (7.1%), "4 times a year" group (22.2%), "2 times a year" group (20%), and the "not applicable" group (4.5%) (graph 13). Relatively speaking one could think that a higher risk exists for infection in the 4 times a year and 2 times a year groups, however the number of infected dogs was very low (2 dogs and 1 dog respectively).

95.6% of the dogs had the anti-ectoparasitic applied to them within the past 3 months (graph 14). In the case of 3 dogs it was applied between 3 to 6 months ago and in the case of 2 dogs the last time was over a year ago. All the infections (10 dogs) were found in the first group, which made out 9.3% of the group. This is the expected number of infections when taking the prevalence into consideration.

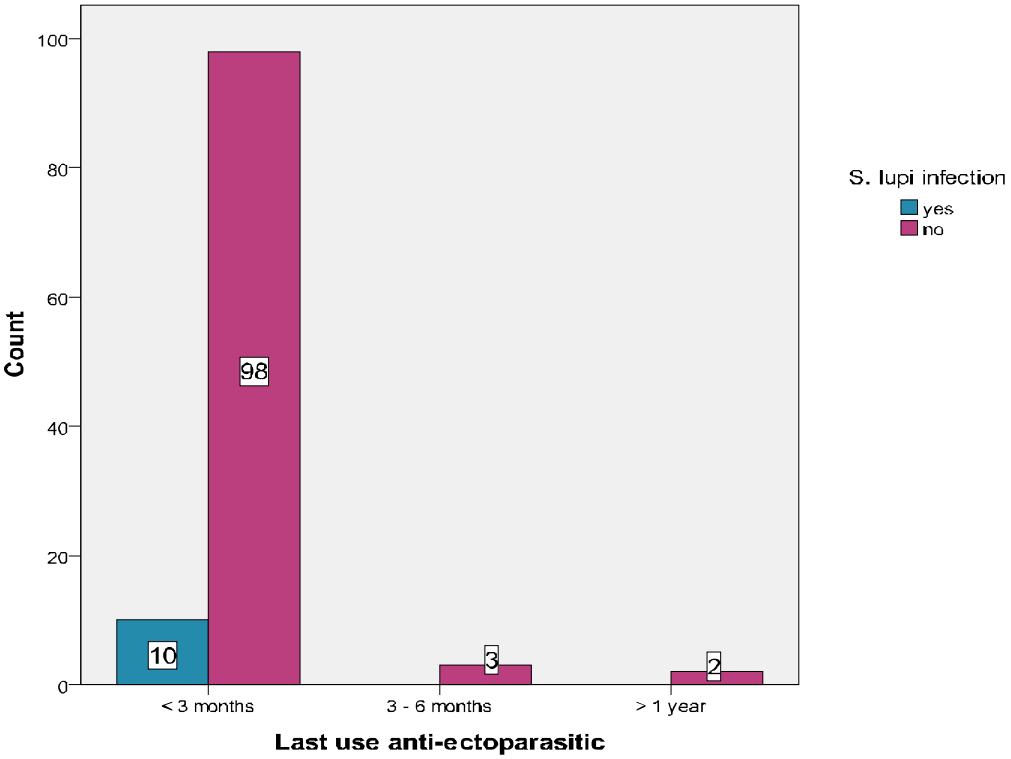
Graph 12 – Use of an anti-ectoparasitic agent and *S. lupi* infections



Graph 13 – Frequency of use of anti-ectoparasitic agent and *S. lupi* infections



Graph 14 – Last use of anti-ectoparasitic agent and *S. lupi* infections



4.1.4 Health state dog

Feeding condition

98 dogs (77.2%) were in a good feeding condition, 15 dogs (11.8%) were considered to be in an excessive state, and 14 dogs (11%) were considered to be in a poor state (graph 15).

In the “good” category 8.2% (8 dogs) had an infection, while in the“excessive” and “poor” categories the percentages were 20% (3 dogs) and 14.3% (2 dogs) respectively (not significant).

Clinical signs

The clinical signs that were found in infected dogs were: vomiting (1dog), diarrhea (1), anorexia (2) and weight loss (1) (table 3). 1 dog showed only weight loss, the second dog showed only anorexia and the third dog showed vomiting, anorexia and diarrhea.

In total 88 dogs did not show any clinical signs, while 41 dogs did show at least one sign. 10 infected dogs (76.9%) fell in the first category, not showing any sign of having an infection. Only 3 of the dogs that showed clinical signs had a *S. lupi* infecton, representing 7.3% of that group.

Of the total of 9 dogs showing the clinical sign vomiting, only 1 dog (11.1%) had a *S. lupi* infection. For the other clinical signs the percentages were: diarrhea (14.3%), anorexia (15.4%), and weight loss (6.3%).

Graph 15 – Feeding condition and *S. lupi* infections

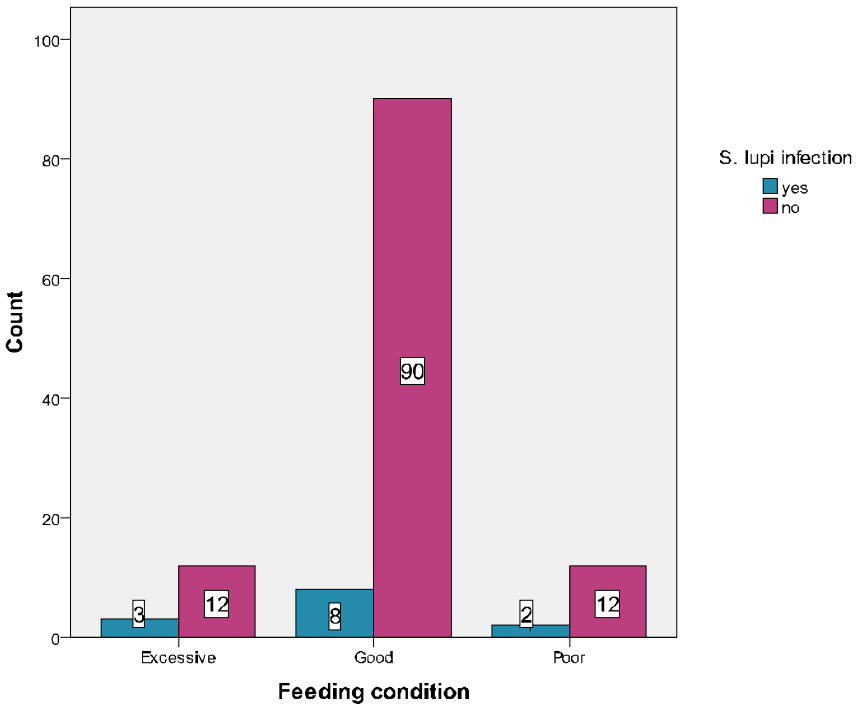


Table 3 – Clinical signs and *S. lupi* infections

	S. lupi infection	
	yes	no
Vomiting	1	8
Regurgitation	0	4
Melena	0	3
Hypersalivation	0	0
Diarrhea	1	6
Dyspnea	0	6
Coughing	0	12
Paraparesis	0	5
Back pain	0	1
Pyrexia	0	4
Weakness	0	6
Anorexia	2	11
Weight loss	1	15
Anemia	0	1

4.2 Other gastrointestinal parasite infections

The same population as described above was examined for the fecal presence of eggs of *Trichuris* sp., *Ancylostoma* sp., *Dipylidium* sp. and *Toxocara* sp.. In total 44 dogs (28%) had a gastrointestinal parasite other than *S. lupi*, of which 38 dogs (86.4%) had a single infection and 6 dogs (13.6%) had a mixed infection. *Ancylostoma* sp. was found the most in both domestic and shelter dogs (table 4). It was found in dual infections with *Trichuris* sp. in 23.6% of the dogs. *Dipylidium* sp. was seen in 2 dogs as a single infection, and in one case in combination with *Ancylostoma* sp. None of the dogs were found shedding *Toxocara canis* eggs.

In 9 dogs (5.7%) *Spirocerca lupi* was present as a single infection. *S. lupi* was seen in a dual infection with *Ancylostoma* sp. in 3 dogs (1.9%), with *Trichuris* sp. also in 3 dogs (1.9%). In only 1 case (0.6%) did a *S. lupi* infection occur together with *Trichuris* sp. and *Ancylostoma* sp.

Table 4 – Infections with intestinal parasites in dogs in Curaçao

	Dog sample	
	Domestic (n = 131)	Shelter (n = 26)
Ancylostoma sp.	29 (22.1%)	4 (15.4%)
Trichuris sp.	12 (9.2%)	2 (7.7%)
Dipylidium sp.	2 (1.5%)	1 (3.8%)

4.3 Necropsy results

Necropsy was performed on 2 male and 2 female dogs. The estimated ages for the males were 1 and 8 years, and 2 and 4 years for the females. Only the 4 year old female was neutered, the other dogs were not. No *S. lupi* infection nor signs of past infections could be found. Also none of the dogs had adult heartworms.

5. Discussion

The *S. lupi* prevalence of 10.2% was expected, since the last time the prevalence has been examined in 1986 (4.5%), no effort had been made in disease prevention and control. Slightly more infections were found when using a loop sample compared to using a spontaneous sample. However, nothing can be said on the sensitivity and specificity of the test using the different samples, since the true infective state of the dogs was unknown.

In previous studies, no sex or age predilection has been found. Therefore, the significant association found between the age group 1-3 years and an infection was not expected. A possible explanation for the higher infection rate in this age group might be because coprophagous behaviour often starts prior to 12 months of age [Hofmeister, E. H. 2003] and pups and juveniles are more likely to display this behaviour [Khoshnegah, J. 2011]. Thereby increasing the risk of acquiring an infection. As expected no association was found between sex or reproductive state and having an infection.

In earlier studies hounds and large breed dogs seemed to be predisposed to an infection [van der Merwe et al., 2008]. In this study no association could be found between breed and an infection. However, the 3 infected pure breed dogs were all of a large breed. Also most infections were found in the category cross breeds, and this category contains dogs of different sizes. It may be that this predisposition does exist, but just was not apparent in the results.

If we consider the possible routes of infection, it is expected that shelter dogs are more likely to be infected because of their less selective way of feeding. However as in the previous study done on Curaçao [Saleh et al., 1988], no such association was seen in this study. It might be possible that hunting and coprophagia are more important in acquiring an infection, and that these behaviors occur in both groups equally.

Eating raw animal products, coprophagy and hunting are three possible ways of acquiring a *S. lupi* infection. No association was found between eating raw animal products and an infection. A possible explanation is that owners might not give the parts of an animal in which the Spirocerca cysts are present like the stomach or crop of a bird [van der Merwe et al., 2008], but instead give muscular tissue (meat). Therefore the owner can answer yes to the question, while in fact no increased risk of acquiring an infection is present.

Even though the infection rate in the coprophagous dogs was higher than the not eating dog feces group, no significant association was found between coprophagy and having an infection. The first factor contributing to this result might be the small sample size. Another factor might be the recollection of the owner, they simply might not remember that their dog ate dog poop in the past. Or they might not own the dog that long, for example in the case of an adopted shelter dog. Also a factor of shame might be involved. The dog owner does not want to attribute the behavior to their dog, because it is considered to be undesirable and unsavoury [Wells,D. L. 2003]. A different possibility is that the owner never witnessed this behaviour.

Hunting is a popular activity among dogs on the island of Curaçao. Examples of animals that are hunted are lizards, iguanas, birds and rats. All these animals are possible paratenic hosts for *S. lupi* [van der Merwe et al., 2008], which is the reason why an association was expected. However, none was found. The small sample size might also be an explanation of this finding. Another factor might be that the owners do not know of or do not recollect hunting behaviour in the past. The parasite can stay alive for several years in the esophagus of the dog [Bailey, 1972], which makes it possible for the dog to have acquired the infection in the past without the owner knowing it. For example in the case of an adopted shelter dog. The two infected dogs in the not hunting group were 8 and 13 years old, which supports the possibility of the owner not knowing of or not remembering this behaviour.

A different way of acquiring a *S. lupi* infection is by scavenging. A dog might eat roadkill or the kill of an other animal, like the neighborhood cat. The owner might not even know of this risk behaviour in the case for example the dog can leave the yard on his own.

The management of owners can also influence the risk of the dog having an infection. The expectation is that when dogs are used as a guard dog, they will be kept outside and loose and therefore the risk increases of acquiring an infection because they have an increased risk of running into a paratenic or intermediate host. While if dogs are kept inside or on a chain, the risk of an infection decreases. It is true that guard dogs are kept mostly outside, however on the island of Curaçao most companion

animals (45.2%) are also kept outside or in 42.8% of the cases both inside and outside. So it appears that the use of the dog does not influence the way the dog is kept that much. This could be the reason why no significant association could be found between the use of the dog and an infection. It seems that dogs kept loose have a higher risk of being infected, since 9/10 infected dogs were kept loose. However, no significant result could be found because the sample of dogs kept on a chain was very small.

The use of a kennel can influence an infection in multiple ways depending how much time the dog spends in the kennel. If they are always in the kennel, the risk of acquiring an infection is expected to decrease. If the dog spends a couple of hours a day in the kennel and the rest of the time outside, the risk is expected to be just as high as for a dog kept both inside and outside. The rates of infections did not differ much between dogs kept in and outside a kennel.

The frequent use of insecticides in the yard should decrease the risk, because the intermediate host (the coprophagous beetle) is eliminated. However if dogs can get outside the yard, this effect is reduced so a higher infection rate is expected. The expected rate of infection is also lower if this insecticide is used more frequent and recently. No significant association was found between using the insecticide, frequency of use and last use and having an infection. A possible explanation for this result might be the small sample size. Also the effect of the use of an insecticide on the intermediate host population might not be that significant. The yard is open for insects to come and go, making the killing of those beetles in the yard a mere opportunity for others to take their place. However, we did see a higher infection rate in the category of dogs using insecticides and able to leave the yard compared to dogs not able to leave the yard. This was based on only 2 infected dogs, so this result must be interpreted carefully.

Not many agents can be effectively used in the treatment of spirocercosis. Studies have shown diethylcarbamazine [Mcgaughey, 1950; Seneviratna et al., 1965], disophenol [Seneviratna et al., 1965], doramectin [Berry, 2000] and ivermectin [Mylonakis et al., 2004] to be effective. In this study we tried to determine which agents were used for deworming and against flees and ticks and whether there was an association with having a *S. lupi* infection. The only products in this investigation with an agent which was proven effective, were Ivomec® (ivermectin) and Heartgard® (ivermectin and pyrantel). Therefore the expectation is that if any association is present, it will be found in these groups. Of course the dosage and frequency of use of these products is just as important for their potential effectiveness. No association could be found between using Heartgard and having an infection. A possibility is that the blood level which is reached is not high enough, because the dosage is not high enough. The dosage of ivermectin in Heartgard is 5.99-11.57 µg/kg, depending on the weight of the dog. The dosage proven effective is 600 µg/kg given subcutaneously. Heartgard is given orally, therefore the biological availability after administration should also be taken into account. The difference is substantial and therefore it is a plausible explanation for the found results. Not enough dogs were treated with Ivomec as a deworming agent, so no conclusions can be drawn. In the cases in which Ivomec was used against ectoparasites, there appeared to be an association with not having an infection. The result was tested not significant, which could be a consequence of the small sample size. The dosing does come close to the therapeutic dosage (0.4-0.5 mg/kg). However also important in the therapy of spirocercosis is the frequency of administration, which also might be a reason why no association could be found.

Most infected dogs in this investigation had a good body condition and did not show any clinical signs. This result is supported in other studies, where it is stated that many *S. lupi* infections are subclinical [Fox et al., 1988]. The clinical signs which were found in infected dogs (e.g. anorexia, vomiting, diarrhea and weight loss) were also seen in other studies [Mazaki-Tovi et al., 2002].

The *Ancylostoma* sp. infection rates found were expected, because in the study done in 1986 hookworm infections were also found often [Saleh et al., 1988]. In this study the rates were lower, which is expected since veterinarians on the island are giving the advice to deworm twice a year. In the previous study a lower *Trichuris* sp. infection rate was found compared to the rate in this investigation. This was not expected. No dogs were found with a *Toxocara* sp. infection, which could be due to the fact that only dogs older than 6 months participated in the investigation and the deworming advice given by the veterinarians.

Because of the very small sample size of dogs on which necropsy was performed, no conclusions can be made. In such a small sample it is expected that no dog can be found with a *S. lupi* or *Dirofilaria immitis* infection.

6. Conclusions

In this investigation we found a prevalence of *S. lupi* infections of 10.2% with CI-95% = [5%;15%]. In shelter dogs this percentage was slightly higher (11.5%) compared to domestic dogs (9.9%). The rates of infections between these two groups did not differ much. Also no association was found when looking at sex, reproductive state or geographical location of the dogs. We did however find a significant association between the age group “1-3 years old” and having an infection. According to the odds ratio, dogs between 1-3 years old were 3.67 times more likely to be infected compared to dogs of different ages. A predisposition for large breeds might exist, but was not clear in this investigation because of the many cross breeds.

No direct association was found when looking at the risk behaviours: eating raw meat, coprophagy and hunting. This does not mean that these behaviours are not important routes of infection. However, other routes which were not investigated in this study might also be of importance.

Also no effect on infection rate was seen in the primary use of dogs, how and where they were kept and the use of a kennel. Logically one may assume that dogs who are always inside or in a kennel have a lower risk of acquiring an infection. The use of insecticides in the yard did not prove to be effective in diminishing the risk, probably because the impact on the intermediate host is not big. No association was seen in deworming and the use of Heartgard, because the active ingredients or the dosage used were ineffective against the parasite *S. lupi*. The use of Ivomec however did seem to have effect on the infection rate, however not enough dogs used this therapy for a significant conclusion.

Most of the *Spirocerca lupi* infections (76.9%) were subclinical infections. The clinical signs observed in case it was not subclinical were anorexia, vomiting, diarrhea and weight loss.

More research is needed, especially in the area of therapy. Also more information is needed on the effect of Ivomec® or another ivermectin containing product as a potential preventative therapy for spirocercosis in dogs.

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Appendix 1

Questionnaire *Spirocerca lupi*

Owner information
Name owner: ….....

Date: .....
Veterinary clinic: .....

Dog information
Name: ….....
Date of birth: ….....
Breed: …..... □ Male □ Female Sterilised: □ Yes □ No

□ Domestic □ Stray
Adress where dog lives / neighborhood where dog was found: .....

Primary use (select only one) □ Companion animal □ Guard dog □ Other: .....

Does your dog ever eat uncooked animal products? □ Yes □ No

Do you ever catch your dog eating dog poop? □ Yes □ No

Do you ever see your dog hunt animals? □ Yes □ No

Do you ever find dead animals in the yard? □ Yes □ No

How is the dog kept? (select all that apply)
□ Inside □ Part inside, part outside □ Outside
□ Kennel □ Chain □ Loose □ Other: ….....

Is the dog able to get outside of the yard? □ Yes □ No

Do you use insecticides in your yard? □ Yes □ No

If yes, please specify:
□ The entire yard □ Part of the yard
Name of the product: ….....
Date of last use: ….....
Frequency of use: ….....

Do you deworm or treat your dog against ectoparasites? □ Yes □ No

If yes, please specify:

Name deworming product: .....
Dosage: ….....
Date of last treatment: ….....
Frequency of treatment: ….....

Name product against ectoparasites: .....
Dosage: ….....
Date of last treatment: ….....
Frequency of treatment: ….....

To be filled in by your veterinarian

What is the feeding condition of the dog? □ Excessive □ Good □ Poor □ Emaciated

What clinical signs does the dog show? (select all that apply) □ None

□ Vomiting □ Regurgitation □ Melena □ Hypersalivation □ Diarrhea
□ Dyspnea □ Coughing
□ Paraparesis □ Back pain
□ Pyrexia □ Weakness □ Anorexia □ Weight loss □ Anemia
□ Other: ….....

Appendix 2

Standard protocol of the CSF-method as used by Utrecht University

1. Make a suspension of the fecal sample in water. Use a mortar in hard samples.
2. Use a tea strain to filter out large parts.
3. Swivel the suspension and pour into a centrifuge tube.
4. Centrifuge for 1 minute at 3000 rotations per minute.
5. Pour out the supernatant by carefully turning the tube 180 degrees.
6. Fill half of the tube with the flotation medium, in this case the sugar solution, and suspend the sediment using a spatula.
7. Fill the rest of the tube with the sugar solution until a meniscus forms.
8. Put a coverslip on top of the meniscus and apply slight pressure on top, so it will stick.
9. Centrifuge again, now for 2 minutes at 300 rotations per minute.
10. Remove the coverslip and place on a slide.
11. Examine the slide underneath a microscope.