Canine filariasis in Salamanca (northwest Spain)

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A survey of dogs in four areas of Salamanca province (northwest Spain) was carried out to determine the prevalence of filariids in the canine population. Blood samples from 293 dogs were tested for the presence of microfilariae, using a modification of the Knott technique. For correct identification of filariid species, blood smears associated with the 43 positive Knott tests were histochemically stained for demonstration of the somatic distribution of acid phosphatase activity. The survey revealed the presence in the northwest of Spain of *Dirofilaria immitis*, *D. repens* and *Dipetalonema reconditum*, with an overall prevalence in Salamanca of 12.3%, 0.3% and 2.1% respectively. The greater prevalence of canine heartworm was found in the irrigated area, where 33.3% of the dogs harboured microfilariae. The relationship between the prevalence of *D. immitis* and mosquito populations, and the influence of host sex and age in this infection, are analysed.

The mosquito-borne heartworm *Dirofilaria immitis* is known to produce pathogenic changes in domestic dogs, and this filarial disease appears to be an emerging zoonosis of public health importance (Soulsby, 1982; Ciferri, 1982). In Spain, the only information about the heartworm situation is the sporadic citing of some cases found after dog necropsy (Jimenez Millan, 1959; Pozo Lora, 1960; Cordero del Campillo, 1974).

Probably, *D. repens*, *Dipetalonema reconditum* and *Dip. dracunculoides* are also present in dogs in Spain, but no surveys have been undertaken until recently (Cordero del Campillo *et al.*, 1980). The broad distribution of *Dip. reconditum* (Newton and Wright, 1956; Lindsey, 1961; Pratt *et al.*, 1981; Walters *et al.*, 1981; Pratt and Corwin, 1984; Martin and Collins, 1985; Owen *et al.*, 1986), the presence of *D. repens* in dogs in Italy (Pampiglioni *et al.*, 1982) and *Dip. dracunculoides* in dogs in Portugal (Azevedo, 1943) support such a notion.

The object of the present study was to obtain information on the filariids present in dogs in the northwest of Spain (Salamanca province) by survey of microfilarial infection. An attempt was also made to find the prevalence of canine dirofilariasis in four ecologically different areas.

MATERIALS AND METHODS

Survey Areas

Canine filariasis surveys were conducted in four areas of Salamanca selected on the basis of the distribution of mosquitoes detailed by Encinas Grandes (1982) (see Table 4). The localities sampled in each area are indicated in Fig. 1; all of them are small villages with agricultural and livestock-rearing activities. The area biotopes and most representative mosquitoes are as follows:

A1, is on the Tormes and Almar rivers in an irrigated agricultural landscape with a high population of *Aedes vexans* and *Ae. caspius*.

A2, is situated on a deforested plain associated with cereal and legume cultivation. The species of mosquitoes are the same as in A1, although the populations of *Ae. vexans* and *Ae. caspius* are very small and have a more focal distribution.

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Fig. 1. Map of Salamanca province showing the location of the four areas studied and situation of rural communities sampled. (see Materials and Methods section.)

A3, is a very extensive plain covered predominantly with holm-oakwoods (*Quercus rotundifolia*) and other oakwoods (*Q. fagineae* and *Q. pyrenaica*) associated with cereal cultivation. The most characteristic mosquito is *Ae. quasirusticus*; but there are many other species depending on the density of woodland or available mammalian hosts.

A4, is a mountainous area, although the localities sampled are situated on a low hill (c. 1000 metres) covered with oakwoods (*Q. pyrenaica*) associated with cereal cultivation. The species of mosquitoes are basically the same as in A3 but with smaller populations, *Ae. punctor* being characteristic of the area.

Survey Technique

Blood (2 ml) from domestic dogs was drawn from the cephalic vein with EDTA-filled evacuated tubes. Samples were taken between 10.00 and 18.00 hours from June to September 1987. Modified Knott tests were performed within one to three days of the survey. Blood was haemolysed with 10 ml of 2% formalin and centrifuged at 1500 rpm for five minutes, the supernatant fluid was decanted, and the sediment was stained with several drops of 1:1000 methylene blue. Wet mounts incorporating the total sediment from each sample were scanned for microfilariae.

For differentiation of microfilariae, blood smears were made from samples which gave a positive Knott test. These films were air-dried, fixed in absolute acetone at 4°C for one minute, and stained for the demonstration of microfilarial acid phosphatase activity using the naphthol AS-TR phosphate method of Barka (Chalifoux and Hunt, 1971). Identification of species was based on the somatic distribution of the acid phosphatase activity as described by Chalifoux and Hunt (1971) and Yen and Mak (1978).

Owners were asked about the history of their dog(s), the breed, sex and age, the use of the animals, and so on. Only autochthonous animals were sampled.

RESULTS

A profile of the canine population surveyed is given in Table 1. The number of rural communities sampled in each area and their locations are shown in Fig. 1. Many dogs were mongrels and were used in working and sporting activities, living outdoors. Both sexes were well represented in the survey (Table 1) and all, females and males, were intact (not spayed or neutered).

TABLE 1
Distribution of surveyed dogs by age and sex for each area sampled

Age (years)	Area 1		Area 2		Area 3		Area 4	
	Males	Females	Males	Females	Males	Females	Males	Females
<1	5	1	3	2	0	0	2	1
1	3	4	5	8	6	9	6	3
2	9	11	4	4	11	12	3	3
3	11	8	10	6	5	7	7	6
4	7	4	6	2	8	8	3	3
5	5	2	0	0	6	3	3	2
6	4	1	2	0	0	6	1	1
7	2	1	1	2	2	3	1	1
8	3	1	0	2	2	0	2	1
9	2	0	1	0	0	3	0	0
10	2	1	1	1	1	0	0	0
>10	0	0	1	0	1	1	0	1
Total	53	34	34	27	42	52	28	22

Microfilariae were detected in 43 of the 293 dogs surveyed, constituting a total filarial prevalence of 14.7%. *D. immitis* microfilariae were found in 12.3%, *D. repens* microfilariae in 0.3%, and *Dip. reconditum* microfilariae in 2.1% of the dog populations surveyed. More detailed information is given in Table 2. No mixed infection was found in any of the dogs. Heartworm had a high prevalence in the provincial area with irrigated riparian agriculture (A1 in Fig. 1) where 33.3% of the dog population surveyed was infected, followed by 6.4% in the provincial area with cereal agriculture in the holm-oakwood landscape (A3 in Fig. 1), which is the only area where *D. repens* was found. In the deforested area with cereal and legume agriculture (A2 in Fig. 1) only 1.2% of the dog population was infected, whereas *D. immitis* microfilariae were not found in the dogs surveyed in the mountainous area (A4 in Fig. 1) where only *Dip. reconditum* was present.

TABLE 2
Prevalence of microfilarial infection in dogs from Salamanca province (northwest Spain)

Area	No. dogs sampled	<i>Dirofilaria immitis</i> prevalence (%)	<i>Dirofilaria repens</i> prevalence (%)	<i>Dipetalonema reconditum</i> prevalence (%)
A1	87	33.3	0.0	2.3
A2	62	1.6	0.0	3.2
A3	94	6.4	1.6	0.0
A4	50	0.0	0.0	4.0
Total	293	12.3	0.3	2.1

The prevalence of *D. immitis* and *Dip. reconditum* in relation to sex is shown in Fig. 2. Of 293 dogs whose sex ratio (m = 157, f = 135) did not differ significantly from 1:1, the male dogs were more frequently infected than females. Of a total of 36 dogs infected with *D. immitis* 24 were males, as were four of the six that harboured *Dip. reconditum*. Analysis of these data by the chi-square method shows that the difference in prevalence between the sexes was not statistically

Fig. 2. Prevalence of *Dirofilaria immitis* (■), and *Dipetalonema reconditum* (▨), in relation to the sex of the dogs sampled.

TABLE 3
Prevalence of infection with *Dirofilaria immitis* and *Dipetalonema reconditum* by age of dogs in Salamanca (northwest Spain)

Age (years)	No. of dogs examined	Dirofilaria immitis		Dipetalonema reconditum	
		No. of infected dogs	Percentage of infected dogs	No. of infected dogs	Percentage of infected dogs
<1	13	0	0	0	0
1	44	0	0	1	2.3
2	58	2	3.5	0	0
3	61	6	9.8	2	3.3
4	40	6	15.0	2	5.0
5	21	8	38.1	0	0
6-10	51	14	27.5	1	2.0
>10	4	0	0	0	0

significant ($P > 0.1$) in *Dip. reconditum* infection, whereas *D. immitis* was significantly ($P > 0.01$) more prevalent among male dogs than females. However, the adjustment for both sex and age showed no statistical difference ($P > 0.1$) between the sexes in heartworm infection.

The association of age with the presence of filariid infection is shown in Table 3, and these data are plotted in Fig. 3, where dogs older than six years are grouped. *D. immitis* microfilariae were most frequently found in dogs more than four years old, and the highest prevalence was in the five-year age group, whereas *Dip. reconditum* was most common in dogs up to four years. Animals younger than two years old did not harbour microfilariae of *D. immitis* while, in contrast, *Dip. reconditum* microfilariae were found in one dog one year old.

Fig. 3. Distribution of microfilarial infections by age of dogs in years. *D. immitis*, (—); *Dip. reconditum*, (---).

DISCUSSION

Although all areas of the province of Salamanca were sampled, more information would have been desirable from the holm-oakwood landscape (A3) and from the mountainous area (A4). Nevertheless, the homogeneous distribution of the mosquito populations in these two areas (Encinas Grandes, 1982) supports the idea that in all localities similar results to those found in the sites sampled could be expected. The dog communities surveyed in the irrigated riparian agricultural and in the deforested cereal-legume agricultural landscapes (A1 and A2) are highly representative.

The enzootic zones of canine heartworm in Salamanca province are associated with the areas of irrigated riparian vegetation, as has been shown in other countries (Pratt *et al.*, 1981; Walters and Lavoipierre, 1984) where there is even an apparent local zoonotic transmission to humans (Ciferri, 1982). According to Walters and Lavoipierre (1984), this kind of agricultural practice has created new mosquito-breeding habitats. In Salamanca the irrigated area has a high density of mosquito species (Encinas Grandes, 1982) where *Ae. vexans* and *Ae. caspius* are specially common. *Ae. vexans*, with a recognized capacity to transmit *D. immitis* (Hendrix *et al.*, 1980), could account for such a high prevalence and even for that observed in the cereal-legume agricultural landscape where this species has a residual population (Table 4). Since *Ae. vexans* was not found in the holm-oakwood landscape (A3), some of the mosquito species shown in Table 4 could also be important heartworm vectors in Salamanca. The absence of *D. immitis* microfilaraemia in the dogs from the mountainous area could be the result of the unfavourable climatic conditions, since temperatures do not remain above those needed for the development of the parasite in the vector for sufficient period of time (Fortin and Slocombe, 1981).

The low prevalence of *D. repens* observed in this survey was not expected; on the contrary, we had assumed the infection to be as frequent as that found in Italy (Pampiglione *et al.*, 1982). The presence in Salamanca of *Ae. caspius*, *Anopheles atroparvus* and *Culex pipiens* (Table 4), all suitable vectors of *D. repens*, would suggest a higher canine prevalence of this infection than that found in this present survey.

TABLE 4

Distribution of mosquitoes by areas in Salamanca province. (Abstracted from Encinas Grandes (1982)).
Extremely rare species and those that do not bite mammals are excluded

Species	Area 1	Area 2	Area 3	Area 4	Observations
<i>An. claviger</i>	△○	△○			□ ?
<i>An. petragani</i>			△○	△○	□ ?
<i>An. plumbeus</i>			△○	△○	□ ?
<i>An. atroparvus</i>	▲●	▲●	▲●	▲●	■
<i>Ae. quasirusticus</i>			▲○	▲○	□ Larval breeding-grounds may be close to villages
<i>Ae. rusticus</i>		△○			□
<i>Ae. refiki</i>		△○			□
<i>Ae. leucomelas</i>		△○			□
<i>Ae. sticticus</i>		△○			□
<i>Ae. cantans</i>		△○			□
<i>Ae. excrucians</i>		▲○			□
<i>Ae. flavescens</i>		△○			□
<i>Ae. punctor</i>				△○	□ Infrequent in the villages where sampling was performed
<i>Ae. berlandi</i>	△○	△○	△○	△○	Breeds in garden trees Bites birds more than mammals
<i>Ae. geniculatus</i>			△○	△○	■ Probably not present in A1-A2 due to their deforestation
<i>Ae. caspius</i>	▲●	△●			■ Very frequent in irrigated zones
<i>Ae. vexans</i>	▲●	△●			■ Very frequent in irrigated zones
<i>Ae. vittatus</i>			△○		□ Only frequent along rocky edges of rivers
<i>Cs. annulata</i>	△○	△○	△○	△○	■
<i>Cx. pipiens</i>	▲●	▲●	▲●	▲●	All populations studies until now in Salamanca have been ornithophile
<i>Cx. theileri</i>	▲●	▲●	▲●	▲●	□

● Maximum of population in summer; ○ maximum of population in spring or end of summer; △ uncommon species or with small populations; ▲ common species with large populations; ■ present in urban zones but not inside buildings; ■ present in urban zones and inside buildings; □ not present in villages; ▲ unidentified presence.

The flea-borne filariid *Dip. reconditum* had a similar prevalence in all areas surveyed with the exception of A3. The absence in the holm-oakwood landscape of dogs harbouring microfilariae of this worm is difficult to explain, since fleas are as abundant as in the other areas sampled. In Salamanca this parasite has a low prevalence and it is less frequent than *D. immitis*, as occurs in Australia (Martin and Collins, 1985), the United States (Pratt and Corwin, 1984) and Canada (Owen *et al.*, 1986).

Analyses of canine sex as a factor involved in heartworm prevalence have yielded contradictory results, and whereas some reports indicate no significant differences between the sexes (Lindsay, 1961; Graham, 1974; Butts, 1979; Martin and Collins, 1985) others have reported a significantly higher prevalence in males (Streitel *et al.*, 1977; Stewart *et al.*, 1979; Selby *et al.*, 1980; Walters and Lavoipierre, 1984), as was found in our survey. In most of these studies, adjustment for the physiological sexual state, age and exposure risk of dogs was not considered; this might be responsible for the recorded difference in infection between the sexes. In this connection Selby *et al.* (1980), adjusting for age and physiological sexual state (intact and spayed females, intact and neutered males), found significant relative risks associated only with intact male dogs, suggesting that such differences could be due to a greater tendency of the males to roam and to their greater use for hunting and sporting purposes. However, Walters

and Lavoipierre (1984), adjusting for the physiological sexual state and also the residential period and exposure, found a greater risk associated with male dogs (neutered and intact) and spayed females, suggesting that the susceptibility to this infection could have a physiological basis. Our results are in agreement with the findings of Selby *et al.* (1980), since adjustment (in the irrigated area A1) for age shows no statistically significant relative risk associated with the sex of the animals (all dogs sampled were intact and had the same risk exposure).

Age as a factor related to an increasing risk of heartworm infection has been considered as an important variable (Lindsey, 1961; Graham, 1974; Selby *et al.*, 1980; Walters and Lavoipierre, 1984), although in some surveys age did not appear to affect prevalence (Thrasher *et al.*, 1968; Martin and Collins, 1985). In our study prevalence was significantly related to the host's age, with a high risk of *D. immitis* infection in the five to 10-year-old groups and a pattern like that found by Graham (1974). Age-prevalence data for *D. immitis* indicate no microfilaraemia in dogs less than two years old or older than 10 years, as was found in other surveys (Lindsey, 1961; Graham, 1974; Selby *et al.*, 1980). It is interesting to note that in these two age groups the risk of occult infection (amicrofilariaemia) is very high (Grieve *et al.*, 1986) which, together with the high prepatency of this infection and the small number of the dogs sampled older than 10 years, may have biased the results.

The frequency of *Dip. reconditum* infection varied in an irregular manner with the age of the hosts, and similar data were found by Graham (1974), although Lindsey (1961) reported rather consistent levels of infection with age. However, in our survey the number of dogs infected with *Dip. reconditum* is insufficient to analyse the age-prevalence relationship.

The lack of surveys on the prevalence of canine filariasis in other zones of Spain makes it impossible to analyse our results within the limits of the country. Extensive surveys in the United States (Graham, 1974; Pratt *et al.*, 1981; Pratt and Corwin, 1984; Walters and Lavoipierre, 1984; Pappas and Lunzmann, 1985), Canada (Owen *et al.*, 1986) and Australia (Carlisle and Atwell, 1984), have shown that canine heartworm is an emerging zoonotic problem that needs special attention even in areas where the prevalence of the infection is lower than that observed in Salamanca. In our opinion more attention should be paid to this parasite in Spain since similar results could be expected in other zones—mainly in those with similar mosquito populations to those of the irrigated riparian agriculture landscape in Salamanca.

REFERENCES

- AZEVEDO, J. F., DE. (1943). On the presence of *Dipetalonema dracunculoides* (Cobbold, 1870) among dog in Portugal. Contribution to the study of its morphology. *Anais do Instituto de Medicina Tropical*, **1**, 105–115 + 10 pp.
- BUTTS, J. A. (1979). Survey of *Dirofilaria immitis* in Mecklenburg County, North Carolina. *Journal of American Medical Association*, **174**, 1088–1089.
- CARLISLE, C. M. & ATWELL, R. B. (1984). A survey of heartworm in dog in Australia. *Australian Veterinary Journal*, **61**, 356–360.
- CHALIFOUX, L. & HUNT, R. D. (1971). Histochemical differentiation of *Dirofilaria immitis* and *Dipetalonema reconditum*. *Journal of American Medical Association*, **5**, 601–605.
- CIFERRI, F. (1982). Human pulmonary dirofilariasis in the United States: a critical review. *American Journal of Tropical Medicine and Hygiene*, **31**, 356–360.
- CORDERO DEL CAMPILLO, M. (1974). Parasitic zoonoses in Spain. *International Journal of Zoonoses*, **1**, 43.
- CORDERO DEL CAMPILLO, M. *et al.* (1980). *Indice Catalogo de Zooparasitos Ibericos*. Madrid, Spain: Servicio de Publicaciones del Ministerio de Sanidad.
- ENCINAS GRANDES, A. (1982). *Taxonomia y Biologia de los Mosquitos del Area Salmantina (Diptera: Culicidae)*. Ediciones de la Universidad de Salamanca. Spain.
- FORTIN, J. F. & SLOCOMBE, J. O. D. (1981). Temperature requirements for developments of *Dirofilaria immitis* in *Aedes triseriatus* and *Aedes vexans*. *Mosquito News*, **41**, 625–633.
- GRAHAM, J. A. (1974). Canine filariasis in northeastern Kansas. *Journal of Parasitology*, **60**, 322–326.
- GRIEVE, R. B., GLICKMAN, V. M. D., BATER, A. K., MIKA-GRIEVE, M., THOMAS, CH. B. & PATRONEK, G. J. (1986). Canine *Dirofilaria immitis* infection in a hyperenzootic area: examination by parasitologic findings at necropsy and by two serodiagnostic methods. *American Journal of Veterinary Research*, **47**, 329–332.

- HENDRIX, C. M., BEMRICK, W. J. & SCHLOTTAHAVER, J. L. (1980). Natural transmission of *Dirofilaria immitis* by *Aedes vexans*. *American Journal of Veterinary Research*, **41**, 1253-1255.
- JIMENEZ MILLAN, F. (1959). Contribucion al estudio de los helmintos parasitos de los animales domesticos. *Revista Iberica de Parasitologia*, **19**, 25.
- LINDSEY, J. R. (1961). Diagnosis of filarial infections in dogs. I. Microfilarial survey. *Journal of Parasitology*, **47**, 695-702.
- MARTIN, T. E. & COLLINS, G. H. (1985). Prevalence of *Dirofilaria immitis* and *Dipetalonema reconditum* in greyhounds. *Australian Veterinary Journal*, **62**, 159-163.
- NEWTON, W. L. & WRIGHT, W. H. (1956). The occurrence of a dog filariid other than *Dirofilaria immitis* in the United States. *Journal of Parasitology*, **42**, 246-258.
- OWEN, J., SLOCOMBE, D. & McMILLAN, J. (1986). Heartworm in dogs in Canada in 1985. *Canadian Veterinary Journal*, **27**, 324-328.
- PAMPIGLIONE, S., FRANCO, F. & CANESTRI TROTTI, G. (1982). Human subcutaneous dirofilariasis. I. Two new cases in Venice. Identification of the causal agent as *Dirofilaria repens* Railliet and Henry, 1911. *Parassitologia*, **24**, 155-165.
- PAPPAS, L. G. & LUNZMAN, A. T. (1985). Canine heartworm in the domestic and wild canids in southeastern Nebraska. *Journal of Parasitology*, **7**, 828-830.
- POZO LORA, F. (1960). Aportaciones al inventario y ecologia de los helmintos espanoles. Especies encontradas en Cordoba. *Revista Iberica de Parasitologia*, **20**, 403-410.
- PRATT, S. E., CORWIN, R. M., SELBY, L. A. & RHOADES, J. D. (1981). Prevalence of *Dirofilaria immitis* and *Dipetalonema reconditum* infections in Missouri dogs. *Journal of the American Veterinary Medical Association*, **179**, 592-593.
- PRATT, S. E. & CORWIN, R. M. (1984). *Dirofilaria immitis* and *Dipetalonema reconditum* in dogs in Nebraska and Missouri in 1984. *Veterinary Medicine and Small Animals Clinician*, **79**, 180-181.
- SELBY, L. L. A., CORWIN, R. M. & HAYES, H. H. (1980). Risk factors associated with canine heartworm infection. *Journal of the American Veterinary Medical Association*, **176**, 33-35.
- SOULSBY, E. J. L. (1982). *Helminths, arthropods and protozoa of domesticated animals*. 7th Edn. New York: Baillière Tindall.
- STEWART, G. L., SMYTH, W. G. & REDDINGTON, J. J. (1979). Canine infection with *Dirofilaria immitis* in North Texas, U.S.A. *Southwest Veterinary*, **32**, 199-202.
- STREITEL, R. H., STROMBERG, P. G. & DUBEY, J. P. (1977). Prevalence of *Dirofilaria immitis* infection in dogs from a humane shelter in Ohio. *Journal of the American Veterinary Medical Association*, **170**, 720-721.
- THRASHER, J. P., GOULD, K. G., LYNCH, M. J. & HARRIS, C. C. (1968). Filarial infections of dogs in Atlanta, Georgia. *Journal of the American Veterinary Medical Association*, **153**, 1059-1063.
- WALTERS, L. L., LAVOPIERRE, M. M. J., TIMM, K. I. & JAHN, S. E. (1981). Endemicity of *Dirofilaria immitis* and *Dipetalonema reconditum* in dogs of Pleasant Valley, Northern California. *American Journal of Veterinary Research*, **42**, 151-154.
- WALTERS, L. L., LAVOPIERRE, M. M. J. (1984). Landscape epidemiology of mosquito-borne canine heartworm (*Dirofilaria immitis*) in Northern California, U.S.A. *Journal of Medical Entomology*, **21**, 1-16.
- YEN, P. K. F. & MAK, J. W. (1978). Histochemical differentiation of *Brugia*, *Wuchereria*, *Dirofilaria* and *Breintia* microfilariae. *Annals of Tropical Medicine and Parasitology*, **72**, 157-162.