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Survey for *Trichinella* spp. in red foxes (*Vulpes vulpes*) in Belgium

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Abstract

Concurrently with a survey for *Echinococcus multilocularis* in the red fox (*Vulpes vulpes*) in Flanders, northern Belgium, serological and parasitological analyses for *Trichinella* spp. were carried out from 1996 to 1999. Muscle samples from foxes in Wallonia, southern Belgium, were obtained during a survey for rabies and alveolar echinococcosis from 1998 to 2000.

In muscle samples from tongue, diaphragm, hindlegs and tail of 179 Flemish foxes no larvae were found by trichinoscopy. Serum and muscle juice of, respectively 176 and 26 animals were examined using an ELISA for the detection of antibodies against excretory–secretory (ES) antigen. There were eight (4.5%) positive sera, but no positive muscle juice samples.

All muscle samples from 639 foxes in Wallonia proved to be negative for larvae in artificial digestion. Serum and muscle juice of 130 and 478 foxes, respectively were examined in ES-ELISA. There were 61 (46.9%) positive sera and 90 (18.8%) positive muscle juice samples. A comparison between 88 serum and muscle juice samples of the same foxes showed that only half of the serum-positive animals were detected using muscle juice. However, for establishing the true meaning of these results, a more profound epidemiological study on the vulpine population in Belgium is necessary.

Keywords: *Trichinella* spp.; Fox; Belgium; Epidemiology-Nematoda

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1. Introduction

Trichinellosis, a zoonotic infection that is caused by *Trichinella* spp., is acquired mainly by the ingestion of infected pork or horsemeat. The prevalence of *Trichinella* spp. in pigs in Europe has well been established for many years (Campbell et al., 1988) and recent outbreaks following the ingestion of raw horsemeat have demonstrated the importance of this source of infection (Dupouy-Camet et al., 1994). On the contrary, prevalence studies in wild animals are scarce. The red fox (*Vulpes vulpes*) is regarded as one of the principle reservoirs (Losson, 1994; van Knapen, 1998), and because it feeds mainly on rodents, it is a main indicator for *Trichinella* spp. in the wild (sylvatic cycle). Geerts et al. (1995) could not demonstrate infection in 118 foxes in Belgium. On the other hand, data from our neighbouring countries included 3.9% positives of 276 foxes in The Netherlands (van der Giessen et al., 1998), 0.026% of 3889 foxes in Germany (Soulé, 1991) and 0.9% of 7138 foxes in France (Pozio et al., 1996). According to Pozio et al. (1996) it also appeared that the infection in foxes was much lower in areas between 0 and 500 m altitude (0.1%) than at higher altitudes (2.5%).

Recent surveys for alveolar echinoccocosis in Flanders and Wallonia, and for rabies in Wallonia, provided the opportunity to study the prevalence of trichinellosis in foxes in a larger sample in Belgium.

2. Material and methods

A total of 818 red foxes were examined for the presence of *Trichinella* spp. Among these were 179 foxes from Flanders (northern Belgium) and 639 foxes from Wallonia (southern Belgium). There were 248 males, 236 females, 62 young animals and 207 adults.

The Flemish foxes were either shot or killed in road accidents in 1996–1999. According to dental inspection, most of these animals were older than 1 year. Because these animals were part of a survey on alveolar echinococcosis, the carcasses were frozen at −80 °C for several weeks prior to necropsy and sampling. Serum samples were extracted from blood cloths and muscle samples were taken from tongue and diaphragm (Kapel et al., 1994, 1995), leg (Kapel et al., 1994, 1995; van Knapen, 1998) and tail (Hinaidy, 1970) for the first 142 foxes. Samples from the anterior tibial muscles (van Knapen, 1998) were selected thereafter. The foxes from Wallonia were autopsied by the Pasteur Institute in Brussels in search of rabies and alveolar echinococcosis in 1998–2000. Fresh muscle samples (anterior tibial muscle) of these foxes were sent to our laboratory together with serum samples.

Similar to Haralabidis et al. (1989) and Kapel et al. (1998), who reported a good correlation between ELISA results of serum and muscle extracts in experimental infections in pigs, extracts of the muscles from some foxes were investigated.

Frozen muscle samples were analyzed by trichinoscopy. From each of the muscle groups, 14 pieces were put in a glass compressorium and examined under a dissecting microscope for the presence of *Trichinella* larvae. Thus, from each fox ca. 1.5 g of muscle in total was examined. Fresh muscle samples were subjected to artificial digestion according to

the guidelines of The Belgian Ministry of Health (IVK, 1995). As such, 100 g of muscle of 10 foxes (10 g per fox) was digested. Serum and muscle juice samples were examined by a modified ELISA for the detection of antibodies against ES antigen from *Trichinella spiralis* larvae (Geerts et al., 1995). Briefly, polystyrene microtitre plates were coated with ES antigen (0.32 μg per well) from *T. spiralis* at 37 °C for 30 min. Plates were washed once with phosphate buffered saline containing 0.05% Tween 20 (PBS-Tw20) and the wells blocked with 150 μl PBS-Tw20 containing 2% new born calf serum (PBS-Tw20-NBCS) and incubated at 37 °C for 15 min. After removal of the blocking buffer, 100 μl sera, diluted 1/200 in PBS-Tw20-NBCS, and muscle juice diluted 1/20, were added and the plates incubated at 37 °C for 15 min. Then plates were washed two times with PBS-Tw20. Anti-dog IgG-peroxidase conjugate was added to the wells diluted 1/5000 in PBS-Tw20-NBCS and incubated at 37 °C for 15 min. After two washings with PBS-Tw20, 100 μl substrate solution (orthophenylenediamine) was added to the wells and incubated at 30 °C for 15 min in the dark. The reaction was stopped by adding 50 μl sulfuric acid (4 N) to the wells. All incubations were done on a shaker, except the incubation of the substrate. Plates were read immediately at 490/655 nm. To determine the cut-off, the Students' *t*-test for comparison of a single observation with the mean of eight negative control samples (Sokal and Rohlf, 1981) was carried out. The ratio, which is the optical density value of the sample divided by the optical density value of the cut-off (at $P < 0.001$), was calculated for each positive result.

3. Results

In none of the muscle samples ($n = 818$) were larvae of *Trichinella* detected, neither by trichinoscopy (Flemish foxes, $n = 179$), nor by artificial digestion (Walloon foxes, $n = 639$).

On the contrary, analysis by ELISA of serum samples detected eight positive samples out of 176 in Flanders and 61 positives out of 130 in Wallonia. Among these 69 seropositive animals were 26 males, 39 females, 4 young animals and 22 adults. Table 1 shows the optical densities. Furthermore, the analysis by ELISA of muscle juice detected 90 positives in Wallonia but none in Flanders. The results of the muscle juice ELISA are shown in Tables 2 and 3.

Table 1
Positive serum ELISA results of Belgian foxes examined for *Trichinella* spp. and parasitologically negative

ELISA ratio[a]	Flanders ($n = 8$)	Wallonia ($n = 61$)	Belgium ($n = 69$)
Median	3.2	2.6	2.7
Total range	1.6–12.0	1.5–22.9	1.5–22.9
1.5–10	7	53	60
10–20	1	4	5
20–30	0	4	4

[a] Ratio = optical density value of the sample/optical density of cut-off at $P < 0.001$.

Table 2
Serum and muscle juice ELISA and parasitological results of foxes in Flanders and Wallonia during a 1996–2000 survey for *Trichinella* spp.

Region	Parasitological technique (no. of positives/no. of foxes)	Serum ELISA no. of positives (%)	Muscle juice ELISA no. of positives (%)
Flanders	Trichinoscopy (0/179)	8/176 (4.5)	0/26
Wallonia	Artificial digestion (0/639)	61/130 (46.9)	90/478 (18.8)
Total	Trichinoscopy/AD[a] (0/818)	69/306 (22.5)	90/504 (17.9)

[a] AD: artificial digestion.

Table 3
Comparison of serum and muscle juice ELISA of 114 parasitologically negative foxes in Flanders and Wallonia during a 1996–2000 survey for *Trichinella* spp.

	Serum-ELISA	Muscle juice-ELISA
Flanders ($n = 26$)[a]	26 (−ve)	26 (−ve)
Wallonia ($n = 88$)	21 (+ve)	21 (+ve)
	22 (+ve)	22 (−ve)
	45 (−ve)	45 (−ve)

[a] There were no corresponding Flemish positive samples.

4. Discussion

Parasitological as well as serological techniques were used to study the prevalence of trichinellosis in the red fox in Belgium. While the presence of *Trichinella* larvae could not be demonstrated in any of the muscle samples, serology of 306 foxes suggested infection with this parasite in 69 animals. The number of examined males and females was almost equal, but there were considerably more positive females. This is contrary to the results of Wacker et al. (1999), who found no difference in the sexes, but his sample size of males was much larger than females. The number of juvenile animals was three times less than that of adults, yet more adult foxes were seropositive, which can be explained by the longer potentially exposure to the parasite (Wacker et al., 1999).

However, correct interpretation of these results requires a fair estimation of the sensitivity of the techniques concerned. Madden and Murrell (1990) reported levels of detection with ES-ELISA, artificial digestion and trichinoscopy of, respectively one larva per 100 gram, one larva per gram and three larvae per gram. Consequently, ES-ELISA is 100 times more sensitive than artificial digestion of muscle samples and 300 times more sensitive than trichinoscopy. Obtaining well preserved and reliable samples from foxes is difficult. Carcasses are sporadically presented for examination and preservation at −80 °C, such as for the Flemish foxes, eliminates the possibility to carry out artificial digestion.

The estimated prevalence of *Trichinella* spp. in foxes in other European regions ranges from 0.1% in regions of France below 500 m altitude (Pozio et al., 1996) to 3.9% in The Netherlands (van der Giessen et al., 1998). Because of this rather low prevalence, a sufficient number of animals are needed in order to allow a solid interpretation of the test results. Considering a sensitivity and specificity of the ES-ELISA of, respectively 96 and 98% (Geerts et al., 1995), the predictive value of a positive test result varies between 4.58 and

66.08% and the predictive value of a negative test result varies between 99.83 and 100%, when the prevalence ranges from 0.1 to 3.9%. This means that positive results have a much higher probability of being false, if the real prevalence should be only 0.1%, even though some of the optical densities reached high levels (Table 1). Given a specificity of 98% for the ES-ELISA, one can expect six false positives out of 306 samples. Cross-reactions with *Trichuris vulpis* could be responsible for false positive reactions, because whipworms share common antigens with *Trichinella* spp. (Murrell et al., 1986). The very high seroprevalence in Wallonia also can be caused partly by overestimation of the transient infections with persisting antibodies (Wacker et al., 1999). Another explanation for the difference between Flanders and Wallonia may be the abundance of wildlife (foxes, wild boar) in the latter. Additionally, the quality of the sera could have influenced the ELISA results.

The serum ELISA results were corroborated by the muscle juice ELISA results. In total 90 out of 504 muscle juice samples (17.9%) were positive (Table 2). According to Kapel et al. (1998), who performed ELISA in experimental infections in pigs, a good correlation exists between results of serum and muscle juice. Comparing the 114 serum and muscle samples of the same foxes, a concordance of 91 out of 114 samples (79.8%) exists. Furthermore, examining the positive Walloon samples (Table 3), only half of the positive serum samples ($n = 43$) are positive in muscle juice ELISA ($n = 22$). The median ratio obtained in the 21 positive serum and muscle juice samples of the same foxes was respectively 5.2 and 2.5, indicating the detection of a much higher amount of antibodies in serum than in muscle. However, a high positive correlation ($r = 0.80$) existed between the ratios of serum and muscle juice. Because all examined foxes were parasitologically negative by artificial digestion, only a small number of larvae per gram could have been present, as has been suggested also for the wild boar in Wallonia (Losson et al., 1995). In our hands the performance of the ELISA using muscle juice was less than using serum. Therefore, contrary to the observations of Kapel et al. (1998), the use of muscle juice might underestimate the real prevalence of trichinellosis.

5. Conclusion

The serological and muscle juice data suggest that trichinellosis exists in the Flemish and Walloon vulpine population. However, demonstration of the parasite in muscle tissue of such sero or muscle juice positive animals is necessary to confirm the ELISA results. Nevertheless, when infections exist, the foxes probably harbour only a small number of larvae. More foxes should be sampled in Belgium to get a realistic estimate of the prevalence of *Trichinella* spp. and to allow a reliable interpretation of the results obtained with ELISA.

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