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Intestinal helminths of red foxes (Vulpes vulpes) in north-west Italy

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Article info

Summary

Received June 11, 2015 Accepted January 14, 2016 A total of 180 foxes (*Vulpes vulpes*) from an area scarcely investigated of north-west Italy, were examined for intestinal helminths using sedimentation and counting technique (SCT). Faecal samples were submitted to centrifugation with 50 % zinc sulphate used as flotation solution.

No fox was found completely negative for intestinal helminths. The most frequently identified nematodes were *Uncinaria stenocephala* (70.0 %), *Molineus legerae* (27.2 %), *Toxocara canis* (26.7 %), *Toxascaris leonina* (25.6 %), *Trichuris vulpis* (21.1 %), *Aonchotheca putorii* (8.9 %), *Pterygodermatites affinis* (5.6 %). Genus *Mesocestoides* (81.7 %), family Dilepididae (29.4 %) and *Taenia* spp. (8.3 %) were the most prevalent cestodes. All foxes were negative for *E. multilocularis* and *E. granulosus*. In two foxes trematodes belonging to the family Plagiorchidae were found.

The study highlighted that foxes are hosts of intestinal helminths of veterinary and medical importance which may be transmitted to dogs and humans.

Keywords: intestinal helminths, Vulpes vulpes, fox, Liguria, Italy

Introduction

The red fox (*Vulpes vulpes*) is the most widespread wild carnivore in the world and is found throughout Italy, in particular in the northern and central areas. This species has adapted to a wide range of habitats and has a highly varied diet, depending on the availability of prey (Larivière & Pasitschniak-Arts, 1996). This opportunistic feeding behaviour has played an important role in the recent colonization of urban and peri-urban areas (Contesse *et al.*, 2004). In fact, in many European countries such areas have seen a dramatic increase in the distribution and density of the red fox over the last two decades (Romig *et al.*, 1999; Eckert *et al.*, 2000; Vervaeke *et al.*, 2005; Veronesi *et al.*, 2014).

Different habitats and diet composition strongly influence the helminth fauna of this wild carnivore (Barbosa *et al.*, 2005; Eira *et al.*, 2006; Hegglin *et al.*, 2007). The red fox is the definitive host of a

wide variety of intestinal helminths of both veterinary and public health concern. The zoonotic species include *Echinococcus multilocularis*, the etiological agent of alveolar echinoccocosis (AE) (Guerra *et al.*, 2014), *E. granulosus*, less frequent in foxes, the etiological agent of cystic echinoccoccosis (CE) (Richards *et al.*, 1995), ascarids and ancylostomids, which are responsible for visceral and cutaneous *larva migrans* syndromes (Richards *et al.*, 1993; Vergles Rataj *et al.*, 2013).

The red fox is also the definitive host of many intestinal parasitic species responsible for minor zoonosis, such as *Trichuris vulpis* (Traversa, 2011), *Mesocestoides litteratus, Mesocestoides lineatus* (Fuentes *et al.*, 2003), *Dipylidium caninum* (Chappell *et al.*, 1990) and other species of the genus *Taenia* such as *T. crassiceps* (François *et al.*, 1998). The fox is also the main reservoir for *Trichinella* spp. (Pozio *et al.*, 1991).

The aim of the present study was to conduct an epidemiological

survey on foxes' intestinal parasites in a previously scarcely studied area of north-west Italy (Liguria and southern Piedmont). The purpose was to evaluate the risk of transmission not only among the foxes, but also to domestic animals (pets and livestock) and humans.

Materials and Methods

Sampling

A total of 180 red foxes (107 males and 73 females) were obtained from 2009 to 2013 in the provinces of Imperia and Cuneo (north-west Italy). Foxes were culled according to the Italian law No. 157/92 and collected by a provincial office of the Veterinary Public Health Services (Section of Imperia of the Experimental Zooprophylactic Institute of Piedmont, Liguria and Aosta Valley, hereafter EZI). Individual data on the area of origin, gender, weight and age were recorded. The age of the animals was estimated on the basis of the general size of the body and of the dental development, as described by Harris (1978). Foxes were classified as "young" (1 year of age or less, n=45) or "adult" (more than 1 year of age, n=135).

Viscera were separated from the rest of the carcass during the necropsy at EZI and then transferred to the Parasitology Section of the Department of Veterinary Sciences, University of Pisa. The results of the examination of the extraintestinal viscera (cardio-pulmonary system, stomach, kidneys, urinary bladder, liver and muscle tissues) are reported in Macchioni *et al.* (2013) and in Magi *et al.* (2015).

Intestinal examination

The intestines were frozen at - 80°C for at least seven days for biosecurity reasons (inactivation of eggs of *E. multilocularis* and *E. granulosus* and of other Taenidae as causes of minor zoonoses). The intestine was then examined with the sedimentation and counting technique (SCT), in accordance with recommended methods for the detection of *E. multilocularis* and other small helminths (Eckert *et al.*, 2001). The intestine was divided into five segments each of which was opened longitudinally. The intestinal content was initially inspected macroscopically to collect large parasites. Each segment was then washed in a conical beaker containing 1 liter of tap water, scraping the mucosal surface in order to collect all the intestinal content. The beakers were then left standing for at least 30 minutes. The sedimentation was repeated until a clear supernatant was obtained. The sediment was then divided into small aliquots in Petri dishes for stereomicroscopic examination.

Coprological examination

Rectal faecal samples (at least 3g) were subjected to coprological analysis to detect parasitic eggs and larvae. Flotation in centrifuge with 50 % zinc sulfate (s.g. 1.350) used as the flotation solution, according to the procedure described by Dryden *et al.* (2005) was utilized.

The specific identification of parasites was based on observation of the morphology of eggs, larvae or adult worms under light microscope and according to the taxonomic keys (Yamaguti, 1959; Campbell, 1991). All parasites found were isolated, counted, separated by gender, and stored in 70 % alcohol.

Statistical analysis

Prevalences with 95 % confidence intervals (CI), mean abundance, mean intensity and range were calculated (Bush et al., 1997). Multiple parasitic infections were also described. For the most prevalent parasites, a negative binomial distribution was fitted to frequency data (number of foxes hosting a number of parasites). The parameters of the fitted distributions are written in terms of the mean number of helminths <x> of a certain species and of the corresponding variance s2 as follows: p=<x>/s2, k =<x>p/(1-p). A 'goodness of fit' chi squared test was carried out in order to compare observed and expected frequency distributions. This was done in order to investigate the ecological equilibrium among hosts and parasites as an indication of overdispersion (Bliss & Fisher, 1953). The results of the coprological tests were compared with necropsy (gold standard). Pearson's chi squared test and Fisher's exact test were carried out to compare parasite prevalences at different age groups and gender classes. The significance of the tests was reached for P values lower than 0.05. The analysis was carried out using Microsoft Excel® and R 2.9.1 (R Development Core Team, 2009).

Results

Intestinal examinations

The results of the examination of the intestine by SCT are shown in Table 1. The nematode species found were: *Uncinaria stenocephala, Molineus legerae, Toxocara canis, Toxascaris leonina, Trichuris vulpis, Aonchotheca putorii,* and *Pterygodermatites affinis.* The most prevalent cestodes belonged to the genus *Mesocestoides*, followed by cestodes from family Dilepididae and from the genus *Taenia*. On the basis of the morphologic and morphometric analyses of the scolex and of proglottids, most cestodes of the family Dilepididae were identified as *Joyeuxiella* spp. Regarding *Taenia* spp., only in four cases was it possible to identify the species on a morphological basis as *T. polyacantha* and *T. pisiformis*. All foxes were negative for *E. multilocularis* and *E. granulosus*. In two foxes trematodes were found and identified as belonging to the family Plagiorchidae. Epidemiological parameters are also shown in detail in Table 1.

Multiple intestinal infections were observed. Intracommunities consisting of three different helminths species were found in $30.9\,\%$ of examined foxes and subsequently two species in $22.4\,\%$, four in $18.8\,\%$, five in $12.1\,\%$, as well as six in $1.8\,\%$ of the foxes. No fox was found completely negative for the intestinal helminths.

Comparing prevalences of intestinal parasites between age classes of the hosts, a significant difference was observed only

Table 1. Results of the intestinal examinations of 180 red foxes by SCT (% = prevalence; CI = 95% Confidence Interval; MA = Mean Abundance; MI = Mean Intensity; R = Range)

Intestinal parasites (180 foxes)	%	CI	MA	MI	R
Nematoda					
Uncinaria stenocephala	70.0	63.3 - 76.7	5.56	7.9	1 – 62
Molineus legerae	27.2	20.7 – 33.7	1.11	4.1	1 – 30
Toxocara canis	26.7	20.2 – 33.1	1.07	4.0	1 – 37
Toxascaris leonina	25.6	19.2 - 31.9	2.00	7.8	1 – 50
Trichuris vulpis	21.1	15.1 – 27.1	0.39	1.9	1 – 9
Aonchotheca putorii	8.9	4.7 – 13	0.69	7.8	1 – 35
Pterygodermatites affinis	5.6	2.2 - 8.9	0.42	7.6	1 – 40
Cestoda					
Mesocestoides spp.	81.7	76.0 - 87.3	44.44	54.4	10 – (~200
Family Dilepididae	29.4	22.8 - 36.1	5.55	18.8	5 – (~110)
Taenia spp.	6.1	2.6 - 9.6	0.20	3.3	3 – 10
Taenia polyacantha	1.1	0 – 2.6	0.03	3.0	1 – 3
Taenia pisiformis	1.1	0 – 2.6	0.02	2.0	1 – 2
Trematoda	1.1	0 - 2.6	0.02	2.0	1 – 3

for Mesocestoides spp. (P value of chi squared test= 0.021) and Pterygodermatites affinis (P value of Fisher's exact test = 0.040) which showed a higher prevalence in adult foxes (> 1 year of age). In fact no significant differences in parasitic prevalences with respect to the gender were found. The presence of intestinal nematodes and cestodes is primarily determined by the fox feeding habits, which do not vary among gender (Artois, 1989; Richards et al., 1995; Vervaeke et al., 2005).

For the most prevalent nematode species (U. stenocephala, M. legerae, T. leonina and T. canis) negative binomial distributions were found to fit the data (all P values of chi squared 'goodness of fit' test were higher than 0.05). The parameters of the fitted distribution were: U. stenocephala k = 0.37, p = 0.117; M. legerae, k = 0.13, p = 0.107; T. leonina, k = 0.108, p = 0.455 and T. canis k = 0.10, p = 0.046. These results indicate an ecological equilibrium between hosts and parasites.

Our study reveals that foxes in the study area host a wide variety of intestinal helminth species.

Coprological examination and comparison with intestinal examination

Table 2 shows the results of the coprological examination of 180 faecal samples by a flotation method. In order to assess the sensitivity (S) and specificity (S') of the coprological test, the results of the coprological tests were compared with the intestinal examination by SCT that is considered as gold standard. While the specificity was high for both nematodes and cestodes (>0.95, with the exception of Ancylostomatidae s'= 0.83), the sensitivity was always lower. Values observed for nematodes were: Family Ancylostomati-

dae S=0.60; *T. leonina* S=0.57; *T. canis* S=0.56; *M. legerae* S=0.51; *P. affinis* S=0.40; *A. putorii*, S=0.38; *T. vulpis* S=0.19. As expected, the estimated sensitivity was particularly low for the cestodes: Family Taenidae S=0.09 and *Mesocestoides* spp. S=0.03.

Discussion

The present study confirms that red foxes in Liguria host many parasite species, as already reported in foxes in Italy and Europe. From a comparison of our results with those reported in other surveys, several observations can be drawn.

Nematodes - Uncinaria stenocephala (prevalence 70.0 %), the dominant intestinal nematode in the present study, is a species commonly encountered in red foxes in many European areas and in the Mediterranean. In the last few years, prevalence around 40 % or higher have been found in different epidemiological studies. For example the prevalence in Slovenia was 58.9 % (Vergles Rataj et al., 2013), in Denmark 54.4 % or 84.1 % respectively (Al-Sabi et al., 2013; Franssen et al., 2014), and in Lithuania 76.9 % (Bružinskaitė-Schmidhalter et al., 2012). Previous studies from the other regions of Italy reported a prevalence of 39.1 % of infected foxes among 129 examined in Tuscany (Magi et al., 2009), and 51.3 % of 645 foxes in northern Italy (and specifically in the regions Aosta Valley, Lombardy, Trentino Alto Adige and Veneto) (Di Cerbo et al., 2008). The relationship between *U. stenocephala* and human cutaneous larva migrans remains unclear. However in one study it was shown that percutaneous infection induced serpiginous tracks which persisted for 3 – 4 weeks (Fülleborn, 1927; Bowman et al., 2010).

Table 2. Results of copromicroscopic examinations of faecal samples in 180 red foxes. The sensitivity (S), specificity (S') of the coprological test was compared with the SCT results

Coproscopy with flotation (180 foxes)	%	CI	S	S'
Nematoda				
Ancylostomatidae	47.2	39.9 – 54.5	0.60	0.83
Toxocara canis	18.3	12.5 – 23.8	0.56	0.95
Molineus legerae	17.8	12.5 -23.8	0.51	0.95
Toxascaris leonina	14.4	9.4 – 19.7	0.57	1.00
Aonchotheca putorii	7.2	3.0 -10.3	0.38	0.96
Trichuris vulpis	4.4	1.7 – 8.0	0.19	0.99
Pterygodermatites affinis	2.2	0.2 - 4.7	0.40	1.00
Physaloptera spp.	1.1	0.0 - 0.28		
Cestoda				
Mesocestoides spp.	3.3	0.9 - 6.4	0.03	0.97
Hymenolepis diminuta	2.8	0.5 - 5.5		
Taenia spp.	0.6	0.0 - 1.7	0.09	1.00

The second most common intestinal nematode was *M. legerae*. Although *M. legerae* is not commonly found in European foxes its presence should not to be considered exceptional as this species is typically associated with wild carnivores (Manfredi *et al.*, 2003). The prevalence reported here (27.2 %) is higher than all values found in the literature previously (Manfredi *et al.*, 2003: 9.8 % of 42 foxes, Segovia *et al.*, 2004: 2.0 % of 399 foxes, Di Cerbo *et al.*, 2008: 2.9 % of 645 foxes). One of the first records for this nematode was in foxes from Belgium and France (Durette-Desset & Pesson, 1987). The geographical distribution of this species in Italy had been so far restricted to the north-eastern districts. Therefore the discovery of *M. legerae* in foxes from north-western Italy suggests that the distribution of this parasite may have extended to the whole Alpine region.

The third most common intestinal helminth was *T. canis* (26.7 %), followed by *T. leonina* (25.6 %). *T. canis* and *T. leonina*, and *U. stenocephala* are among the most frequently encountered nematodes of red foxes in Europe. Variable values for the prevalence of *T. canis* are reported in the literature. 60 % prevalence have been found in Denmark (Al-Sabi *et al.*, 2013: , Saeed *et al.*, 2006), 38.3 % in Slovenia (Vergles Rataj *et al.*, 2013), 40.5 % in Lithuania (Bružinskaitė-Schmidhalter *et al.*, 2012) and 29.4 % in Romania (Barabási *et al.*, 2010). The prevalence in Italy varied from 9.1 % (Magi *et al.*, 2009) to around 50 % (Di Cerbo *et al.*, 2008) .

The prevalence of *T. leonina* in this study was higher than in most of the surveys presented in literature. In Slovenia a value of 2.5 % was found among 428 foxes examined (Vergles Rataj *et al.*, 2013) and lower values were reported in Denmark (Saeed *et al.*, 2006: 0.6 % of 1040 foxes) and in Romania (Barabási *et al.*, 2010: 4.6 % of 561 foxes). However, in Switzerland a prevalence of 37.3 % was found among 228 foxes examined (Reperant *et al.*, 2007). Since the transmission of *T. leonina* is mainly linked to the ingestion of

a paratenic host (small mammals, birds, invertebrates) (Reperant *et al.*, 2007) the prevalence observed in the study area could be due to the existence of these components in the diet of the foxes examined. This hypothesis is also supported by the high prevalence of *Mesocestoides* spp., which are typically linked to a predatory diet.

Although most human infections remain asymptomatic, *T. canis* is a neglected zoonotic disease responsible for the visceral and ocular larva migrans syndrome. It mainly affects children, especially those from socio-economically disadvantaged populations existing both in the tropics and in industrialized nations (Genchi et al., 1988; Macpherson, 2013). Toxascaris leonina occurs in dogs, cats and various wild canids and felids throughout the world and is considered to have limited zoonotic potential (Morgan, 2013). Foxes may play a significant role in the transmission of this zoonosis (Richards et al., 1993; Reperant et al., 2007; Brochier et al., 2007). Trichuris vulpis was found in 21.1 % of the foxes. A similar value (27.2 %) was found by Barabasi et al. (2010) in Romania, while lower values (0.7 %) were found in Slovenia (Vergles Rataj et al., 2013) and in Bulgaria (Kirkova et al., 2006: 12.2 % of 113 foxes). Lower prevalences (0.2 %) were found in other Italian studies (Manfredi et al., 2003; Di Cerbo et al., 2008). The zoonotic potential of *T. vulpis* is considered notable (Traversa, 2011).

Aonchotheca putorii was found in the intestine and also in the stomach (see Magi et al., 2015). Although this Trichuridae nematode typically infects mustelids, it has also been found in other wild and domestic hosts (Segovia et al., 2004). In Italy it was reported for the first time by lori et al. (1990) in foxes from Trentino Alto Adige and Latium, with prevalences of 3.4 % and 1.1 %, respectively, and subsequently it was found by Manfredi et al. (2003). Prevalences reported in European studies vary from 1.2 % (399 foxes examined in Spain, Segovia et al., 2004) to 29.4 % (310 foxes

examined in Lithuania, Bružinskaitė-Schmidhalter *et al.*, 2012). *Pterygodermatites affinis* (5.6 %) is a nematode encountered in wild carnivores. In Europe it has been found in foxes from the Iberian Peninsula (Eira *et al.*, 2006, Segovia *et al.*, 2004, Gortázar *et al.*, 1998) and in France (Pètavy, 1990: 5 % of 150 foxes). Recently this parasite has also been reported in Tunisia (Lahmar *et al.*, 2014) and Saudi-Arabia (Alagaili *et al.*, 2011). In Italy this species was reported for the first time by Iori and Leto (1990). All subsequent reports (Stancampiano *et al.*, 1998; Capelli *et al.*, 2003; Manfredi *et al.*, 2003; Di Cerbo *et al.*, 2008) found this parasite in north-east Italy. Meanwhile we found it in the north-west. This data thus suggest a wide distribution across the alpine and sub-alpine regions.

Cestodes - Mesocestoides spp. were found to be the most prevalent (prevalence 81.7 %) and abundant (mean abundance ~50) cestodes and the dominant intestinal helminths in the study area. These cestodes are commonly found in European foxes with variable prevalences (Eira et al., 2006). The prevalence found in the present work is among the highest and is similar to the values found in Lithuania (Bružinskaitė-Schmidhalter et al., 2012: 78.4 % of 269 foxes), in Poland (Borecka et al., 2009: 71.2 % of 639 foxes), in Spain (Gortazar et al., 1998: 71.6 % of 415 foxes) and in Greece (Papadopoulos et al., 1997: 73.2 % of 314 foxes). Lower values have been reported in Romania (Barabasi et al., 2010: 28.7 % of 561 foxes), in Slovenia (Vergles Rataj et al., 2013: 27.6 % of 428 foxes), in Slovakia (Hrčkova et al., 2011: 41.9 % of 3175 foxes) and from the other studies in Italy (Di Cerbo et al., 2008: 27.4 % of 645 foxes; Magi et al., 2009: 45.5 % of 129 foxes). The complete life cycle of *Mesocestoides* spp. is not known. Oribatid mites probably act as first intermediate hosts, while reptiles, amphibians, birds and small mammals are second intermediate hosts. All these vertebrates are potential prey for the foxes. The high prevalence of Mesocestoides spp. found suggests these intermediate hosts are important components in the fox predatory diet, as it was already observed for some nematodes.

Mesocestoides spp. have a zoonotic potential. At least 27 human cases have been reported to date in Japan, China, Korea, United States, Rwanda and Greenland (Fuentes et al., 2003). The infections were due to eating habits that includes consumption of raw or undercooked snakes, chicken and wild game viscera (Fuentes et al., 2003).

Cestodes belonging to the family Dilepididae in the present study were found with an overall prevalence of 29.4 %. Based on the morphological and morphometrical analyses of scolexes and proglottids most Dilepididae were identified as *Joyeuxiella* spp., while *Dipylidium caninum* was not found. This was similar to the survey of Segovia *et al.* (2004) where three different Dilepididae species were present, but *D. caninum* was not found. This latter species can be distinguished from the other Dilepididae by typical egg capsules which can contain up to 30 eggs, while the egg capsules of other

species contain a single egg (Euzéby, 1961). Specimens of *Taenia pisiformis* (1.1 %), *Taenia polyacantha* (1.1 %) and unidentified *Taenia* species (6.1 %) were found. The presence of *T. pisiformis* suggests that lagomorphs (hares, rabbits), that represent the intermediate hosts (Guerra *et al.*, 2013), may be infected in the area, entailing significant economic losses. Both these Taenidae species have been previously found in foxes in Europe (Barabasi *et al.*, 2010; Segovia *et al.*, 2004; Shimalov & Shimalov, 2003; Gortazar *et al.*, 1998).

Echinococcus multilocularis was not found in this study with SCT, which is considered to be the gold standard by O.I.E. To date a few cases of *E. multilocularis* found in Italy have been reported only in foxes from Trentino Alto Adige (Manfredi *et al.*, 2002; Casulli *et al.*, 2005). *E. granulosus*, which has never been found in Italian foxes except for some immature stages in Sardinia (Leoni *et al.*, 1986), was not found in this study.

Trematodes - Trematodes were found in two foxes. Specimens were identified as Plagiorchidae. However, due to bad sample preservation, it was not possible to accomplish specific identification by morphology. Intestinal flukes in foxes are rare in Italy and have been reported only by Di Cerbo et al. (2008) and Manfredi et al. (2003). Reports regarding trematodes occur more frequently in European studies outside Italy.

Faecal examination – As expected the present study confirms the very low sensitivity of coprological examinations for tapeworm diagnosis and also shows low sensitivity values for the nematodes. This was already observed by Martini and Poglayen (1990). The highest sensitivity values were found for Ancylostomatidae (S=0.60) and ascarids (S=0.56 for *T. canis* and S=0.57 for *T. leonina*). These values are similar to those reported by Wolfe et al., (2001). A very low sensitivity for *T. vulpis* (0.19) was found.

The present study corroborates the frequent occurrence of false negative results from coprological examinations, while false positive results may be due to the ingestion of food contaminated with parasite eggs (i.e. pseudo-parasitism). The false negative results are possibly due to the small amount of faeces that can be recovered from foxes' intestines or as a result of intermittent eggs excretion. Additionally some helminth eggs (ascarids and ancylostomids) in frozen faeces can modify their shape (Schurer et al., 2014; Guerra et al., 2013). As detected by coprological examinations the low prevalence of *T. vulpis* may be complicated by the fact that eggs can be disguised with those of other Trichuridae, such as *Eucoleus aerophilus, Eucoleus boehmi* and *A. putorii* (Magi et al., 2012; Veronesi et al., 2014).

This implies that a single coprological examination of a subject may not be reliable and should always be repeated, using an appropriate flotation solution. In addition, coprological methods such as flotation may be coupled, when possible, with molecular diagnostic methods (Guardone *et al.*, 2013).

Conclusions

The present epidemiological study on intestinal helminths showed that the fox, in this scarcely studied area, hosts a wide range of helminth species of medical and veterinary importance. The absence of *E. multilocularis* and *E. granulosus* is notable. The most frequent parasites encountered are nematodes belonging to the families Ancylostomatidae, Ascarididae and Trichuridae, and cestodes belonging to the families Mesocestoididae and Dilepididae, most of them with a zoonotic potential.

The prevalence of zoonotic parasites in foxes in Liguria suggests the need to continue in the surveillance of these helminth species due to the increased proximity of foxes to humans and to domestic dogs with a significant public health implications (Deplazes *et al.*, 2004; Mackenstedt *et al.*, 2015). In fact, foxes can cause public health problems and transmit parasites directly to humans through soil contamination with eggs, or indirectly by infecting intermediate hosts and then domestic animals (Richards *et al.*, 1993; Eckert *et al.*, 2001).

Considering that all parasitic species found in foxes are shared by dogs, the present epidemiological survey is also a source of valuable data on the epidemiology and diagnosis of parasitic species that are less known or probably underestimated in pets.

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Conflict of interest statement

None.

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