

RESEARCH ARTICLE

## Helminthiasis in dogs of University of Peradeniya premises: a potential public health problem

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**Abstract:** A sizable population of stray dogs are wandering in the premises of the University of Peradeniya and they ramble and defecate inside the faculty buildings and student hostels. This may pose a public health risk due to wide range of zoonotic parasites strays might harbour. Here we carried out a cross-sectional, coprological survey to assess the canine helminthiasis in the Peradeniya university premises. Fresh faecal samples were collected from March to June 2018 and were analyzed using a modified Sheather's sucrose flotation technique. Sixty dogs were sampled, of which 76.6% was infected with one or more enteric helminths with a higher prevalence in stray dogs (88.6%) than owned dogs (60.0%). Seven helminth genera were recorded: *Toxocara* (26.7%), *Spirocerca* (26.7%), *Strongyloides* (10.0%), *Trichuris* (5.0%), *Dipylidium* (3.3%), *Capillaria* (3.3%) and *Ancylostoma* (73.3%). All seven genera were recorded in the stray dogs while *Trichuris* was not recorded in the owned dogs. *Ancylostoma* was the most prevalent infection with the highest intensity (103.4 ±198.4 Eggs per gram: EPG). There was no difference in the prevalence of *Ancylostoma* infections between strays (60.0%) and owned dogs (46.7%) but the intensity of infection was higher in owned dogs (155.2 EPG; range 2 - 755 EPG) than strays (71.6 EPG; range of 1 - 546 EPG), irrespective of de-worming. It could be due to development of antihelminthic resistance as routine use likely to accelerate the development of resistance in canine helminths. Except for *Capillaria*, other six helminths recorded were zoonotic, with a potential of humans acquiring the infections when there is close contact with dogs. Stray dogs may act as reservoirs of these infections for owned dogs and humans and *vice versa*. In order to control the zoonotic infections, the stray dog population in and around faculties and residential halls, has to be controlled implementing strict rules on feeding strays inside the university premises.

**Keywords:** Canine helminthiasis, zoonoses, public health.

### INTRODUCTION


Dogs have been a human companion for millennia and their significant impact on human life renders the risk for transmission of zoonotic pathogens (Schantz, 1994). There is a 1:8 ratio of dog to human population in Sri Lanka (De Alwis, 2000), accounting for a large stray dog population. Dogs, especially the strays, are competent reservoir hosts of several zoonotic pathogens. Canine helminths such as *Toxocara canis*, *Capillaria*, *Uncinaria*, *Strongyloides stercoralis* and *Spirocerca lupi* transmitted through soil

contaminated eggs and larvae have a significant impact on both animals and humans (Surgan,1980; Traub *et al.*, 2002; Traversa *et al.*, 2014; Zanzani *et al.*, 2014). These parasites have a cosmopolitan distribution throughout tropical and subtropical regions (Bethony *et al.*, 2006; Mandarino-pereira *et al.*, 2010; Mateus *et al.*, 2014; Zanzani *et al.*, 2014). Following ingestion of eggs or larvae, or skin penetration of larvae, these will develop into adult worms inside the host's gastrointestinal system or other systems like respiratory or circulatory system. Eggs laid by the female worms will pass out with faeces of the host and remain stable for days, for example in hookworms approximately 3 to 84 days under moist humid conditions until the larvae find a suitable host (Brooker *et al.*, 2006; George, 2017). Some hookworms ( e.g. *Ancylostoma duodenale*) can undergo hypobiosis to avoid harsh environmental conditions such as vast temperature changes (Brooker *et al.*, 2006) and can stay dormant for long periods.

Helminth infections can cause severe clinical signs like anaemia, intestinal obstruction, chronic dysentery, epigastric pain, flatulence etc. (Stephenson *et al.*, 2000; Wani *et al.*, 2010; Vercruyse *et al.*, 2011), that eventually lead to the death of the infected animal. *Toxocara canis* and *Ancylostoma* are the primary nematodes that affect dogs throughout the world (Traversa *et al.*, 2014). Importantly, adult females can easily transmit the infection by environmental contamination with infective eggs, mainly contaminated soil. They may harbor somatic larvae which can activate during pregnancy and/ or lactation. Puppies can get easily affected via milk e.g. *Toxocara*. Hence the close association between the bitch and puppy can easily transmit the infection to puppy from the bitch (Traversa *et al.*, 2014). Soil moisture and appropriate humidity can influence the development of eggs and larvae in the outside environment (Brooker *et al.*, 2006). The moisture conditions make eggs more viable and infective, while rainfall patterns provide suitable moisture conditions. Hence the transmission of helminth infection is high during rainy season (Gunawardena *et al.*, 2004).

Helminth infection in humans had been a heavy burden in Sri Lanka during early 1900s'and bi-annual treatment with antihelminthic drugs to children aged 3-18 years for decades, together with the implementation of proper excreta disposal methods, reduced the prevalence substantially. A national survey of school children reported

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6.9% were infected (Pathmeswaran *et al.*, 2010) which is lower than the threshold of 20% prevalence to implement mass de-worming programs (WHO, 2006). However, a higher prevalence of 29% was reported in plantation community (Gunawardena *et al.*, 2011) but this was much less compared to 90% recorded in early 1990s (Sorensen *et al.*, 1996). Currently, the helminth infections of humans are confined to areas with poor sanitary conditions (De Silva, 2014). Studies have shown that stray dogs harbour a wide variety of parasites and may play a role as they can act as a reservoir harboring certain zoonotic helminths to humans (Perera *et al.*, 2013). A recent more comprehensive study analysed GI parasites of dogs and humans in a socioeconomically challenged estate community and also soil samples from the environment providing important insights into one health approach to potential canine zoonoses (Bandaranayake *et al.*, 2019). Among the canine helminths recorded in Sri Lanka, *Ancylostoma caninum*, *Ancylostoma braziliense* and *Toxocara canis* are the most prevalent zoonoses (Senadhira, 1967a, 1967b; Dissanaikie, 1961, 1995; Kannathasan *et al.*, 2012).

Sri Lanka has a large population of stray dogs which are more robust and show tolerance and resistance against many diseases. It is possible that the stray dogs are sub-clinically infected and may provide a continuous source of infection for humans, pedigree dogs and wildlife. In 2006, with the lobbying of animal activists, a presidential order was passed to implement a “no-kill policy” for the canine strays in Sri Lanka involving “catch-neuter-vaccinate-release” method (CNVR). This is a more humane approach and has significantly reduced the number of rabies cases over the years. However, many complaints have been allegedly made to local councils about the stray dogs in public places. The major contributory factors for increasing stray dogs include dumping unwanted puppies in public places, dumping garbage on the streets and excessive breeding of pedigree dogs. The number of stray dogs in the university premises has increased over the years posing a risk to the public, students and staff that reside in the area. Here we investigated helminths harboured by the stray and owned dogs in the premises of the University of Peradeniya to assess their potential as sentinels of parasites of humans.

## MATERIALS AND METHODS

### Study site

University of Peradeniya is located in the central hills of Sri Lanka, where elevation ranges between 473.5 m to 588.5 m. The university spreads across 7 km<sup>2</sup>, along the lower slopes of Hantana Mountain range. Climatically university has a mild climate with an annual rainfall of 2000 mm and an annual mean temperature of 28 °C. The current student population in the University of Peradeniya is about 13,000 of which over 5,000 students are in residential halls located within the university premises. Using a convenient sampling method, sites were selected in the premises of the faculties: Agriculture, Arts, Medicine, Science and Veterinary Sciences as well as five student residential halls: Ramanathan, Hilda Obeysekera, Sangamitta, Wijewardana and Jayathilaka. In addition, university staff quarters

in Rajawatta and Mahakanda areas were also sampled. Although the samples were collected from these specific places, these dogs wander in other places of the university as well.

### Study animals

Both stray and owned dogs were sampled including both males and females as well as adults and puppies. Information on age (whether adults or puppies) and gender of each dog were recorded while collecting the samples. Owned dogs that were de-wormed 2-4 times a year were considered as regularly de-wormed. Stray dog samples were collected from the faculties and halls of residence while samples from the owned dogs were from the staff quarter at Rajawatta and Mahakanda areas.

### Sample collection

A total of 60 dogs were sampled to represent approximately 50% of the dog population in the area. Soon after defecating, a fresh faecal sample was hand-picked into an inverted plastic bag and placed in a cooler. Sample collection was carried out from early morning during 6.00 to 9.00 a.m. for a four month period from March to June 2018. Subsequently, the samples were brought to the Parasitology Research Laboratory in the Department of Zoology, Faculty of Science, University of Peradeniya within 1-2 h from the collection sites and were stored in a refrigerator at 4 °C for 1-5 days until processed.

### Microscopic analysis of helminth eggs

The samples were analysed using the modified Sheather’s sucrose floatation method as previously described (Perera *et al.*, 2013; Jenkins *et al.*, 2017). Briefly, 3 g of sample was measured and was mixed with 15 ml of distilled water. Following centrifugation (at 1370 g for 20 min), the supernatant was discarded. Subsequently, the tube with the pellet was filled with Sheather’s sugar solution and centrifuged. Following several centrifugation steps the remaining pellet with the suspension (~ 0.5 ml) was observed under the stereo microscope. The helminth eggs observed under high power (40x10) were identified using both morphology and morphometry according to Taylor *et al.* (2007). In addition, the number of eggs in 0.5 ml was counted and the number of eggs per gram (EPG) of each faecal sample was calculated as previously described (Perera *et al.*, 2013), assuming that the eggs in all 3 g of faeces have been concentrated into the final pellet of 0.5 ml as a result of several centrifugation steps. Identification based on egg morphology and morphometry was further confirmed by the identification of larvae obtained by larval cultures.

### Larval cultures

Larval stages were identified by preparing larval cultures. Fresh faeces were mixed with sterile soil and was placed in a glass beaker. The sample was moistured with a small amount of water, kept the beaker upside down in a Petri dish and the Petri dish was filled with water. The beaker was kept for 3-4 days under sunlight and then water samples were examined under the light microscope for larvae.

## Statistical Analysis

Infection prevalence and intensity were calculated and were compared between owned and stray dogs using a Chi square test and Mann Whitney U test, respectively using SPSS statistical software version 25.0.

## RESULTS

Faecal samples from 35 stray dogs and 25 owned dogs were collected from the University of Peradeniya premises (Figure 1).

### Prevalence of parasites

Of 60 dogs 76.6% were infected with one or more helminth species. Between the two categories of dogs, stray dogs had a higher prevalence of infection (31/35; 88.6%) compared to that of owned dogs (15/25; 60.0%; Chi square test  $\chi^2=22.134$ ;  $p < 0.0001$ ). Overall, female dogs had a significantly higher prevalence (27/35; 77.1%) compared to male dogs (16/25; 64%;  $\chi^2=4.063$ ;  $p=0.044$ ) but there was no difference in the prevalence of infection between puppies (< 12 months; 9/12; 75%) and adults ( $\geq 12$  months; 35/48; 72.9%;  $\chi^2=0.104$ ;  $p=0.747$ ).

### Parasite diversity and intensity

Seven helminth genera were recorded (Table 1) of which the most common infection was *Ancylostoma* (44/60; 73.3%), followed by *Toxocara* (16/60; 26.7%) and *Spirocerca* (16/60; 26.7%; Figure 2). All seven genera were recorded in the stray dogs while *Trichuris*, was not recorded in the owned dogs (Figure 2). All helminth genera had a higher intensity of infection (EPG) in stray dogs compared to that

of owned dogs, except for *Ancylostoma* (Table 2). Although there was no difference in the prevalence of *Ancylostoma* infection between owned dogs (60.0%) and stray dogs (46.7%; Chi square test  $\chi^2= 3.39$ ,  $p=0.6539$ ), owned dogs had a higher intensity of *Ancylostoma* (155.2 EPG) than strays (71.6 EPG; Mann Whitney U test;  $U=190.5$ ;  $p=0.041$ ) and between adults (65.5 EPG) and puppies (242.2 EPG;  $U=87$ ;  $p=0.041$ ). However, there was no difference in the intensity of *Ancylostoma* infection between females (110.8 EPG) and males (98.4 EPG;  $U=213.5$ ;  $p=0.865$ ).

*Toxocara* infection showed the second highest infection intensity in both owned (62.7 EPG) and stray dogs (48.7 EPG) but there was no significant difference in *Toxocara* infection between the two groups (Mann Whitney U test;  $U= 25.5$ ;  $p=0.522$ ). Further, there was no difference in the *Toxocara* intensity of infection between females (65.7 EPG) and males (27.3 EPG;  $U=16.0$ ;  $p=0.097$ ) and between puppies (145.0 EPG) and adults (37.9 EPG;  $U=15.5$ ;  $p=0.060$ ). For *Spirocerca*, there was no difference in the intensity of infection between stray dogs (31.1 EPG) and owned dogs (18.6; EPG; Mann Whitney U test;  $U=22.5$ ;  $p=0.562$ ) or between puppies (48.3 EPG) and adults (15.8 EPG;  $U=15.0$ ;  $p=0.246$ ). Among the observed parasite genera *Dipylidium*, *Toxocara*, *Stroglyoides*, *Trichuris* and *Ancylostoma* have zoonotic potential while *Spirocerca* has been listed as a very rare zoonotic disease.

## DISCUSSION

The study shows that the dogs in the Peradeniya university premises harbour many helminth infections and the prevalence of infection is higher in stray dogs than owned



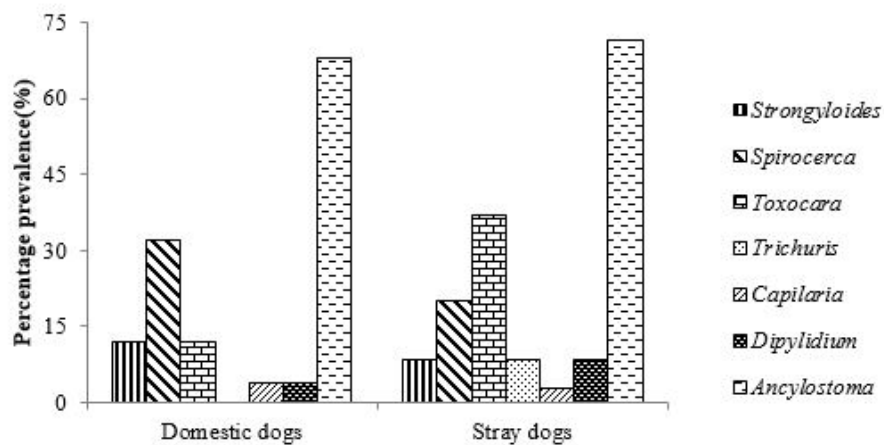
**Figure 1:** Map of Peradeniya University showing the sample collection sites (red dots).

**Table 1:** Prevalance of helminthes among the males and females and adults and puppies of the stray and owned dogs.

Helminth	Overall Prevalence (%)	Stray dogs (35)(%)				Owned dogs (25)(%)			
		Gender		Age		Gender		Age	
		Male (10)	Female (25)	Adult (33)	Puppy (2)	Male (15)	Female (10)	Adult (15)	Puppy (10)
<i>Strongyloides</i> sp.	10.0	-	8.6	11.4	-	4	4	-	8
<i>Spirocerca</i> sp.	26.7	-	17.1	22.9	5.7	12	12	20	4
<i>Toxocara</i> sp.	26.7	14.3	22.9	34.3	2.9	4	8	8	4
<i>Trichuris</i> sp.	5.0	5.7	2.9	8.6	-	-	-	-	-
<i>Capilaria</i> sp.	3.3	2.9	2.9	5.7	-	4	-	4	-
<i>Dipylidium</i> sp.	3.3	-	11.4	11.4	-	-	4	4	-
<i>Ancylostoma</i>	73.3	31.4	51.4	74.3	2.9	32	28	40	20

**Table 2:** Intensity of infection of helminthes among the males and females and adults and puppies of the stray and owned dogs.

Helminth	Overall Intensity Of Infection (EPG)	Stray dogs (EPG ± SD)				Owned dogs (EPG ± SD)			
		Gender		Age		Gender		Age	
		Male	Female	Adult	Puppy	Male	Female	Adult	Puppy
<i>Strongyloides</i> sp.	7.7 ± 4.5	-	10.0 ± 3.6	8.3 ± 1.5	15.0	3.0	3.0	-	3.0
<i>Spirocerca</i> sp.	24.5 ± 28.1	43.3 ± 50.5	22.0 ± 23.6	19.7 ± 20.1	100.0	21.3 ± 22.3	16.0 ± 21.5	11.2 ± 15.8	31.0 ± 24.4
<i>Toxocara</i> sp.	51.3 ± 81.3	32.6 ± 53.8	58.7 ± 99.6	42.2 ± 83.5	126.0	1.0	93.5 ± 99.7	12.0 ± 15.5	164.0
<i>Trichuris</i> sp.	3.7 ± 4.6	1.0	9.0	3.7 ± 4.6	-	-	-	-	-
<i>Capillaria</i> sp.	4.5 ± 4.9	-	1.0	1.0	-	8.0	-	8.0	-
<i>Dipylidium</i> sp.	10.3 ± 11.3	-	26.0 ± 13.3	8.7 ± 13.3	-	-	15.0	15.0	-
<i>Ancylostoma</i> sp.	103.4 ± 198.4	9.5 ± 5.5	99.2 ± 177.1	54.17 ± 121.9	280.5 ± 375.5	200.9 ± 284.2	96.4 ± 215.3	96.0 ± 247.7	231.3 ± 259.6

**Figure 2:** The prevalence of enteric parasites among owned and stray dogs of University of Peradeniya premises.



dogs. The problem is greater in the free roaming dogs due to more frequent exposure of animals to faeces, and the condition is exacerbated during warm, rainy seasons when temperatures and humidity are increased. Studies show that effect of dog breed is not significant in helminth transmission, where prevalence and intensities of parasites were higher in crosses of local breeds compared to exotic breeds. Similar parasitic prevalences have been recorded in both pure and mixed breeds (Oliveira-Sequeira et al., 2002; Senlik et al., 2006; Aydenizöz-Özkayhan et al., 2008; Perera et al., 2013). Factors like gender of dog, associate environment, nutritional levels, anthelmintic treatment and immune status can affect the prevalence of helminth infections considerably (Bugg et al., 1999; Oliveira-Sequeira et al., 2002; Scaramozzino et al., 2009).

Stray dogs can be found all over the university premises and are mostly vaccinated against rabies. However, the lack of de-worming and defecation in public areas contribute considerably to the transmission of infections to other dogs as well as to humans. The most common helminth infection was hookworm, *Ancylostoma*. A previous study carried out in Hantana area in the Kandy district show high *Ancylostoma* burden in dogs (Perera et al., 2013). *Ancylostoma* sheds large number of eggs per week (Traversa, 2012; Traversa et al., 2014). The transmission of hookworm eggs are mainly controlled by climatic conditions, as eggs require warm and moist conditions to develop into larval stages (Sorensen et al., 1994). Heavy infections cause diarrhoea and blood mucus passes with faeces and may also develop severe compensate anaemia (Rep et al., 1971; Carroll and Grove, 1984; Traub, 2013). Four hookworm species are known to infect dogs: *Ancylostoma caninum*, *A. braziliense*, *A. ceylanicum* and *Uncinaria stenocephala* (Landman and Prociw, 2003; Palmer et al., 2008) of which *Ancylostoma* spp. primarily occur in moist and warm climatic zones whereas *U. stenocephala* prefers temperate and subarctic regions (Levine, 1980; Bowman, 2009). Among these, *A. caninum* is one of the most pathogenic of dogs (Bowman et al., 2003) and is also a zoonotic species (Prociw and Croese, 1996). These three species of *Ancylostoma* have been reported from Sri Lanka (Seneviratna, 1955; Dissanaikie, 1961; Kannangara and Karunaratne, 1970). In fact, *A. ceylanicum* was first described from a civet cat in Sri Lanka (Loos, 1911) and later shown to have a high prevalence of 92% in dogs and occurred in greater numbers than other species of *Ancylostoma* (Kannangara and Karunaratne, 1970). *Ancylostoma ceylanicum* is zoonotic and is recorded in humans in India, West New Guinea, Taiwan and the Philippines (Choo et al. 2000). In the present study, both owned dogs and strays were equally infected with *Ancylostoma* but the intensity of infection was higher in the owned dogs, irrespective of deworming. Generally, dogs respond quickly to anthelmintics and faecal egg counts decrease over time (Nolan et al., 1992). It is recognised that routine use of anthelmintic drugs likely to accelerate the development of resistance in canine helminths as in livestock helminths (Besier and Love, 2003; Moncayo et al., 2008). Currently, however there are limited reports on anthelmintic resistant nematodes of dogs (Raza et al., 2018).

The second most prevalent canine helminth was *Toxocara* and *Spirocerca* infections. *Toxocara* eggs can survive for a long period of time in outside harsh environmental conditions due to the thick protective egg shell with several layers (Mandarino-pereira et al., 2010). Infection occurs when embryonated eggs are ingested. Similar to *Ancylostoma*, the larval development of *Toxocara* is determined by soil type, temperature and moisture conditions (Okulewicz et al., 2012). A previous study recorded that *T. canis* is more prevalent in puppies than in adults (Iddawela et al., 1986). Heavily infected pups may continuously whine, shriek and adopt a particular posture by overlapping hind limbs while standing or walking (Raza et al., 2018). *Toxocara canis* has been recorded in both stray and domestic dogs in previous studies conducted in Sri Lanka (Perera et al., 2013) as well as in many other countries like Nigeria (Ugbomoiko et al., 2008), Malaysia (Mahdy et al., 2012), Poland (Borecka, 2005), Portugal (Mateus et al., 2014), Serbia (Ilić et al., 2017), Australia and other Asian countries (Gordan et al., 2017). Human toxocarasis is present almost all over the world and is the most common zoonotic parasitic infection from pets (Shantz, 1994; Aydenizöz-Özkayhan et al., 2008). It manifests as a number of syndromes including visceral, ocular and subclinical forms causes ocular larva migration in humans (Shantz, 1994; Okulewicz et al., 2012). *Spirocerca* infection was also common in dogs in the University premises. Spirocercosis in dogs is caused by the nematode *Spirocerca lupi*. Typical clinical signs are regurgitation, vomiting and dyspnoea. The life-cycle involves an intermediate coprophagous beetle and a variety of paratenic hosts. Larvae follow a specific migratory route, penetrating the gastric mucosa of the host, migrating along arteries, maturing in the thoracic aorta before eventually moving to the caudal oesophagus (Merwe et al., 2008). Here the worm lives in nodules and passes larvated eggs. Early diagnosis of infection is still a challenge and to date no ideal regimen for prophylaxis has been published. *Spirocerca lupi* has been listed as a very rare zoonotic disease (Goldsmid, 2005).

The dogs also harboured *Trichuris*, *Stroglyoides*, *Capillaria* and *Dipylidium* at low prevalences. *Strongyloides* infection is highly prevalent in tropical countries and the parasite has significant zoonotic potential (Raza et al., 2018). Larvae are also passed through milk to puppies. It appears that young dogs, especially puppies are more prone to developing clinical strongyloidosis, and *S. stercoralis* infection was associated with the death of a 10-week old puppy in a kennel (Dillard et al., 2007). *Trichuris* was found in adult stray dogs only. It is an ubiquitous parasite all over the world in kennelled, household and shelter dogs (Traversa, 2011). Eggs are difficult to eliminate and serve as a constant source of infection once mixed with soil, and continually expose dogs to re-infection. Thus, the incidence and parasite burden of trichuriasis is higher in adult dogs compared to younger animals (Raza et al., 2018). The second factor that supports the higher prevalence in adult dogs is the absence of trans-mammary or trans-placental routes of transmission (Schantz, 1999). Parasitic infection would be expected to be higher in strays

where there is contaminated soil in dog environment. *Capillaria* is an infection with non specific clinical signs, which occur through contaminated food and water and the worm resides in the nasal passage, respiratory tract or the bladder causing nasal, pulmonary and urinary disorders. It usually is not transferable to humans (CDC <https://www.cdc.gov/parasites/capillaria/>). All the above infections were nematode while *Dipylidium* was a tapeworm. Infection is acquired by ingestion of infected fleas (Ilić *et al.*, 2017).

Except for *Capillaria*, other six helminths recorded were zoonotic, with a potential of humans acquiring the infections when there is close contact with dogs. The stray dogs usually do not show symptoms of disease but may act as reservoirs of these infections. Therefore, it is important to raise awareness that people are at risk of exposure as these dogs carry potentially hazardous zoonotic pathogens.

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#### DECLARATION OF CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

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