ABSTRACT
Animal Assisted Interventions (AAIs) programmes have been considered useful in different settings, such as hospital, therapeutic, educational and assisted living environments. In these contexts, all animals, and particularly dogs, should be subjected to appropriate health controls to prevent a potential risk of transmission of zoonotic agents. Domestic dogs are reservoirs of many zoonotic pathogens including several gastrointestinal parasites (protozoa and helminths). Therefore, the aim of the present study was to investigate the presence of the protozoan *Giardia duodenalis* and zoonotic gastrointestinal nematodes (geohelminths) in dogs hosted in a dog educational centre in the city of Naples (southern Italy) where the animals were trained to AAI. Between April and June 2016, 74 dog faecal samples were analysed using the FLOTAC dual technique to detect *G. duodenalis* cysts and other parasitic elements. Out of the 74 faecal samples examined, 18 (24.3%; 95% CI = 15.4–35.9) were positive for parasitic elements. Specifically, 8 were positive for *G. duodenalis* (44.4%; 95% CI = 22.4–68.7). In addition, some co-infections were also found: one sample (5.6%; 95% CI = 0.3–29.4) resulted positive to both *Toxocara canis* and *Trichuris vulpis* and two samples (11.1%; 95% CI = 1.9–36.1) were positive to both *G. duodenalis* and Ancylostomidae. Given that children, young adults and immunocompromised individuals are among the main users of the AAIs, specific guidelines targeting *G. duodenalis* and other gastrointestinal zoonotic parasites should be formulated in order to develop effective control and prevention strategies and reduce the zoonotic risk favoured by the human-dog interaction.

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Introduction
In order to control zoonotic diseases naturally transmitted between vertebrate animals and humans, in 2004, the international scientific community used the term ‘One Health’ to define the need for a multidisciplinary approach including human and veterinary medicine, and environmental sciences in Public Health (Chalmers and Dell 2015). Animal Assisted Interventions (AAIs) programmes represent current and concrete examples of One Health, as they involve many health care figures who work in team for the welfare of people, considering the animal (i.e. a co-therapist dog) as a referent of the therapeutic process (Menna et al. 2016). The human-animal interactions, particularly with dogs, have been associated with positive effects on human health and wellbeing (Fine 2010; Menna et al. 2012). The AAIs approach includes animal-assisted therapy (AAT), animal-assisted activities (AAA) and animal-assisted education (AAE); these three activities can produce therapeutic, motivational and educational benefits in people of all ages, particularly among children and the elderly, and in different kinds of patients, from those with physical ailments to those with mental disorders such as dementia and depression (Pedersen et al. 2011; Menna 2016). Consequently, AAIs programmes have been considered useful in different settings, such as hospital, therapeutic, educational and assisted living environments (Banks and Banks 2002; Reed et al. 2012), hence becoming very important in public health care system as a complementary intervention to conventional therapies and activities. In this context, it should be noted that whenever there are animals not subjected to appropriate health control, there could be a serious risk of transmission of zoonotic (infective or parasitic) agents.

Domestic dogs are important reservoirs of many zoonotic pathogens including several gastrointestinal parasites (Robertson and Thompson 2002; Paul et al. 2010). The parasitic risks for humans are mostly posed...
by environmental faecal contamination (Rinaldi et al. 2006). The presence of eggs on the ground is not only implicated with the direct infection for humans but could represent a source of contaminations for pet coats. Indeed, some studies confirmed that the presence of parasitic elements (e.g. embryonated ascarid eggs or Giardia cysts) on the fur of dogs may be considered as a source of human infections via hand-to-mouth contact (Traversa et al. 2014). Eggs, larvae, cysts, and oocysts excreted via the canine faecal route can survive and be infective in the environment over a long time and under different conditions (Rinaldi et al. 2006). Dog faeces deposited on soil in city parks or gardens represent not only an inconvenience, but also can be a health threat (Traversa et al. 2014). Although the canine geohelminths (Toxocara canis, Ancylostoma caninum and Trichurus vulpis) are well-known recognised zoonotic parasites, in recent years major attention have been paid to the protozoan Giardia duodenalis which is now considered the intestinal parasite with a high zoonotic potential among domestic carnivores (Macpherson 2013; Ryan and Cacci 2013; Zanzani, Di Cerbo et al. 2014) as well as the leading cause of parasitic gastroenteritis worldwide (Minetti et al. 2015). One of the limits of research about G. duodenalis in humans is the entity of the risk, in fact, still now, it is not well known (Bouzid et al. 2015). The situation in dogs is more clear with prevalence of G. duodenalis ranging from 1.3% to 24.8% (Epe et al. 2010; Zanzani, Gazzonis et al. 2014). Therefore, the aim of the present study was to investigate, for the first time in Italy, the presence of G. duodenalis and zoonotic gastrointestinal nematodes in dogs involved in AAI.

Materials and methods

Study area

The study was conducted in a dog educational centre in the city of Naples (southern Italy) where the animals were trained to AAIIs through an educational programme according to the guidelines of the Italian National Educational Sports Centre (CSEN). From April to June 2016, a total of 74 faecal samples was collected from owned dogs used in AAI. The information for each dog was collected through an interview performed on arrival at the Centre using a semi-structured questionnaire addressing some generic characteristics (age, sex, and breed of the dogs) and different questions regarding their health status. The dogs were classified into two Age groups, one containing animals from two to five years (n=38) and one containing animals from six to ten years (n=36); two Sex groups, male (n=32) and female (n=42); two Breed groups, crossbred (n=36) and purebred (n=38). All the animals were natives from the Campania region of southern Italy which extends over an area of 13,590 km², mainly hilly, and extends from 0 to 1,890 m above sea level. The climate is Mediterranean with dry summers and rainy winters.

Faecal sampling and FLOTAC technique

Two grams of faeces were placed into the Fill-FLOTAC (Cringoli et al. 2013), a plastic kit to weight, dilute, homogenise and filter the sample. Once in the lab, 18 ml of water (dilution ratio = 1:10) were added to the fresh faeces contained in the Fill-FLOTAC. The suspension was then thoroughly homogenised using the homogeniser stick of the Fill-FLOTAC. The faecal suspension was filtered through the Fill-FLOTAC and 6 ml of the filtered suspension were placed and centrifuged into two conic tubes. After centrifugation (3 minutes at 1500 rpm) the supernatant was discarded leaving only the sediment (pellet) in the tube.

Copromicroscopic examinations were performed using the FLOTAC dual technique (Cringoli et al. 2010) for the detection of helminth eggs and protozoan cysts. This technique is based on the use of two flotation solutions: Sodium Chloride (specific gravity - s.g. = 1200) and Zinc Sulfate (s.g. = 1200). The analytic sensitivity of the FLOTAC dual technique was 2 cysts/eggs per gram (CPG/EPG) of faeces (Cringoli et al. 2010).

Results

Out of the 74 faecal samples examined during the study, 18 were positive for any parasitic element (24.3%; 95% CI = 15.4-35.9) (Table 1). Out of the 18 positive samples the higher prevalence values were found for Giardia (44.4%) and T. vulpis (38.9%). Some co-infections were found during the copromicroscopic investigation: specifically, one sample was positive to both T. canis and T. vulpis (5.6%; 95% CI = 0.3-29.4) and two samples were positive to both G. duodenalis and Ancylostomidae (11.1%; 95% CI = 1.9-36.1). The dogs were not evaluated for the infestations by ectoparasites.

Discussion

This is one of the first parasitological studies in Italy in dogs that participate in Animal Assisted Interventions. Even if these animals were treated usually once a year with large-spectrum antiparasitic drugs by the owners,
the presence of four zoonotic parasites (nematodes and protozoa) in these animals suggest that dogs involved in AAI could play an important epidemiological role in the transmission of parasitic infections to humans.

Dogs that participate in AAI, commonly interact with humans whose immune systems are not functioning optimally (Lefebvre et al. 2008; Kamioka et al. 2014) and several studies show that chronic *Giardia* infection occur in children and immunocompromised individuals (Thompson 2004; Robertson et al. 2010).

The dog is one of the pet closest to the human and the transmission of zoonotic agents is also favoured given the close relationship of human beings with their pet (Feng and Xiao 2011).

According to Bouzid et al. (2015) what risk such endemic colonisation poses to human health is still unclear as it will depend not only on prevalence rates but also on what assemblages of *Giardia* are excreted and how people interact with their pets. In fact, the level of risk depends strictly on the presence of *Giardia*-human assemblages (A and B) (Cacciò et al. 2005). In addition, recent data show the case of a dog that has been associated exclusively to the A assemblage, suggesting the existence of a potential zoonotic reservoir for this assembly (Minetti et al. 2015).

Unfortunately, one of the limits of our study is that assemblages of *G. duodenalis* have not been determined by molecular investigations.

Considering that children, young adults and immunocompromised individuals are among the main users of the AAI, the animals involved should also undergo a specific parasitological diagnosis targeting *Giardia* spp. and other intestinal nematodes (Lefebvre et al. 2008; Silveira et al. 2011). It would be useful to control and reduce the presence of these parasites, also preventing the human transmission where there is an increased risk of infections, such as in childcare centres and day-care centres (Cordell 2001). Therefore, monitoring dogs for zoonotic parasites is necessary for the development and implementation of effective control and prevention strategies that mitigate the burden of zoonotic diseases on Public Health. Prevention through specific guidelines including suitability of patients, animals and infection control policies, need to be formulated before the initiation of AAI and this is the most important way to avoid human dog infections.

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### Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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**Table 1. Parasitological results in dogs involved in Animal Assisted Interventions in southern Italy.**

| Parasites                  | No. of positive | Prevalence (%) | 95% Confidence Interval | CPG/EPG (min-max) |
|----------------------------|-----------------|----------------|-------------------------|-------------------|
| *Giardia duodenalis*       | 8               | 44.4           | 22.4-68.7               | 10–30,000         |
| *Trichuris vulpis*         | 7               | 38.9           | 18.3-63.9               | 2–154             |
| *Toxocara canis*           | 3               | 16.7           | 4.4-42.3                | 50–1260           |
| Ancylostomidae             | 1               | 5.6            | 0.3-29.4                | 32                |

*CPG/EPG = cysts/eggs per gram of faeces.*
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