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An integrated approach to control Cystic Echinococcosis in southern Italy

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ABSTRACT

Cystic echinococcosis (CE) is a severe zoonosis, caused by the larval stage of the tapeworm *Echinococcus granulosus*. This helminth infection is of increasing public health and socio-economic concern due to the considerable morbidity rates that cause economic losses both in the public health sector and in the livestock industry. Control programmes against *E. granulosus* are considered long-term actions which require an integrated approach and high expenditure of time and financial resources. Since 2010, an integrated approach to control CE has been implemented in a highly endemic area of continental southern Italy (Campania region). Innovative procedures and tools have been developed and exploited during the control programme based on the following strategies: i) active and passive surveillance in livestock (using geospatial tools for georeferencing), ii) diagnosis in dogs (using the FLOTAC techniques and molecular analysis), iii) targeted treatment of farm dogs (using purpose-built confinement cages), iv) early diagnosis in livestock (by ultrasonography), v) surveillance in humans (through hospital discharge records analysis), vi) monitoring the food chain (analysing raw vegetables), vii) outreach activities to the general public (through dissemination material, e.g. brochures, gadgets, videos, virtual reality). Over eight years, the integrated approach and the new strategies developed have resulted in a noteworthy reduction of the parasite infection rates in livestock (e.g. up to 30 % in sheep). The results obtained so far highlight that using a one health multidisciplinary and multi-institution effort is of pivotal importance in preparing CE control programmes at regional level and could be extended to other endemic Mediterranean areas.

1. Introduction

Cystic echinococcosis (CE), also known as hydatidosis, is one of the most widespread zoonotic parasitic diseases, caused by the larval stages of *Echinococcus granulosus*, the small dog tapeworm. The life cycle of *E. granulosus* includes canids as definitive hosts and a wide range of domestic and wild mammals, as well as humans, as intermediate hosts. The World Health Organization (WHO) includes human CE in the list of the 20 neglected tropical diseases (NTDs) and in the list of the priority neglected zoonotic diseases (NZDs) for which effective control efforts are needed (WHO, 2020a). In fact, Disability Adjusted Life Years (DALYs) resulting from human CE have been calculated as 184,000, similar to Dengue, Chagas disease and African trypanosomosis (Torgerson et al., 2015).

As for the veterinary sector, the economic and animal health repercussions linked to CE in livestock are mainly due to reduced yield and quality of meat, milk and wool, reduced birth rate and delayed performance and growth, reflecting a global livestock industry loss up to $3 billion, annually (Torgerson and Macpherson, 2011; WHO, 2020b).

Causative agents of CE are members of the *E. granulosus sensu lato* (*s. l.*) complex, which include biological genotypes with differences concerning morphology, host specificity, biochemical parameters, developmental biology and geographical distribution (Romig et al., 2015). Based on recent studies on nuclear and mitochondrial genes,

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E. granulosus complex has been grouped as *E. granulosus sensu stricto* (*s.s.*) (genotypes G1, G2 and G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* (G6/G7, G8 and G10) and *E. felidis* (Maksimov et al., 2020; Vuitton et al., 2020). The taxonomic status of *E. canadensis* is still disputed (Romig et al., 2015) and recently the genotypes G6/G7, G8 and G10 have been proved to be phylogenetically distinct and could be regarded as two distinct species (Laurimäe et al., 2018; Thompson, 2020).

CE is widespread in central Asia, South America and the Mediterranean countries, mainly in rural areas, where cohabitation of dogs, sheep and humans is a common practice (Craig et al., 2015; Deplazes et al., 2017). Eggs shed by infected dogs are the most important source of infection for humans and other intermediate hosts (Deplazes et al., 2017). Therefore, controlling this parasitic infection in animals is crucial to reduce the incidence of human disease (Otero-Abad and Torgerson, 2013). Although control initiatives were started in the second half of the 19th century and have been implemented in selected countries or regions (Craig et al., 2017; Tamarozzi et al., 2020), CE still remains a global concern with high rates of infection both in animals and in humans. A comprehensive review of the global distribution of CE in definitive and intermediate hosts has been recently published (Deplazes et al., 2017), offering the potential to improve the spatio-temporal targeting of control measures and to enhance the cost-effectiveness of integrated control programmes. CE is widespread in south and southeast Europe (Deplazes et al., 2017), with values of prevalence significantly higher in the Mediterranean regions. Italy, especially the southern continental and insular regions (e.g. Basilicata, Campania, Sardinia and Sicily), records the highest prevalence values in humans and animals among the European countries (Deplazes et al., 2017; Varcasia et al., 2020).

As far the Campania region of southern Italy, high CE prevalence values in ruminant livestock have been reported as follows: 10.4 % in cattle (Rinaldi et al., 2008b), 10.5 % in water buffaloes (Capuano et al., 2006), and 51.2 % in sheep (Cringoli, unpublished data); furthermore, a 16.2 % prevalence of *E. granulosus* infection in dogs in the rural areas of the region was reported (Musella et al., 2010). The fertility rate of hydatid cysts in ruminant hosts revealed that sheep carried more fertile cysts (17.1 %) than buffaloes (13.2 %) and cattle (0.0 %), in line with other similar studies (Thompson, 2017). A fine-scale geospatial analysis confirmed the main role of sheep in the transmission of CE in the Campania region, presumably because free-ranging canids with access to infected sheep carcasses might contaminate cattle/water buffalo farms with *Echinococcus* eggs (Cringoli et al., 2007). The primary role of the domestic cycle (sheep-dog) for the transmission of CE in this region was also supported by the findings of the *E. granulosus s.s.* genotypes G1 and G3 in both intermediate (sheep, cattle and water buffaloes) and definitive (dog) hosts (Capuano et al., 2006; Rinaldi et al., 2008a; Maurelli et al., 2018). However, recently, *E. granulosus s.l.* genotype G7 has also been reported in wild boars of the Campania region (Laurimäe et al., 2019; Sgroi et al., 2019).

In order to slow down the transmission of *E. granulosus*, since 2010 the Campania regional government have boosted and financed "EchinoCamp", a control programme aimed at developing an integrated approach to control CE in animals and in humans. For this purpose, different institutions including universities, public research institutes, veterinary and public health authorities have been involved in a multi-institutional approach. The programme is divided in two temporal phases, with different tasks based on livestock surveillance at slaughterhouses, early diagnosis in dogs and sheep, targeted deworming of dogs and outreach activities.

This paper describes the main activities and findings of the EchinoCamp control programme, highlighting the new strategies of action developed and validated in this CE endemic region of southern Italy. The final goal was to develop an integrated approach to support control programmes for CE to be extended to other Mediterranean areas endemic for CE.

2. Material and methods

2.1. Study design

The EchinoCamp control programme was launched in 2010 and divided in two temporal phases. The activities of the first phase were performed from 2010 to 2015 and provided baseline data followed by intervention measures to control CE in the Campania region of southern Italy. From 2016 until 2018, the second phase of the programme was carried out with some implementation of the control measures.

The main tasks of the two phases are summarized in the sections below. Specifically, the activities of EchinoCamp Phase 2 are described in detail, highlighting the new strategies of action developed and validated.

2.2. EchinoCamp - Phase 1

The main tasks used in this phase were:

(i) *Selection of sheep farms to include in the programme.* Given the lack of CE prevalence data in sheep from slaughterhouses, the Regional Observatory for Food Safety - Veterinary Epidemiological Observatory, Istituto Zooprofilattico del Mezzogiorno provided data on cattle/water buffalo farms positive to CE in the Campania region. Thereafter, through the use of the buffer generation function of Geographical Information Systems (GIS), sheep farms to include in the study were selected. Specifically, circular buffer zones of 5 km radius were constructed around each cattle and water buffalo farm positive to CE. Then, within each buffer zone, five sheep farms were selected *at random*.

(ii) *Praziquantel treatment and diagnosis of echinococcosis in shepherd dogs.* The dogs present in each sheep farm selected were treated with praziquantel (5 mg/Kg) and subjected to parasitological examination 24 h post-treatment using the protocol developed by Maurelli et al. (2018).

(iii) *Monitoring of sheep at slaughterhouses*. Active surveillance was conducted each month in sentinel abattoirs to detect CE cysts in sheep of the Campania region.

(iv) *Hospital discharge records (HDRs) analysis*. HDRs data for the six-year period 2010–2015 were provided by the Italian Department of Health and were analysed.

(v) *Information, dissemination and health education for dogs' owners, farmers, school-age children and physicians*. In order to promote adopting healthy practices, dissemination and communication activities were organized with dogs' owners, farmers, school-age children and physicians. For this purpose, educational materials (brochures, posters and gadgets) were designed and distributed to raise awareness and implement recommendations to control CE.

2.3. EchinoCamp – Phase 2

The results obtained during the first phase were analyzed and some critical points were identified and considered in the development of the second phase in order to strengthen the control programme. Specifically, the following implemented tasks were introduced in EchinoCamp Phase 2:

(i) *Treatment and confinement of all dogs for 24–48 h* through a specifically designed and purpose-built modular mobile cage (EchinoCage).

(ii) *Improvement of the safety of laboratory staff* during the diagnosis of *E. granulosus* in dogs, through a specifically designed and purpose-built diagnostic hood (EchinoHood).

(iii) *Collection of data from ruminant (sheep, goats, water buffaloes and cattle) intermediate hosts* to better understand the epidemiological chain of CE.
(iv) *Improvement of the in vivo diagnosis of CE in sheep* by ultrasound (US) technique.
(v) *Monitoring of the food chain* through the analysis of vegetables, for self-consumption or for sale from the sheep farms surveyed.
(vi) *Improvement of health education* and outreach activities through the introduction of new tools such as the virtual reality (VR).

Fig. 1 summarizes the tasks of the two phases divided for each action, highlighting the relevant differences.

2.3.1. Selection of sheep farms

A total of 297 contiguous sheep farms located in a pilot area of the Campania region, highly endemic for CE, were selected for the study; 173 of them had an history of positive animals for CE detected during slaughtering. The location of the sheep farms included in the control programme was geo-referenced using a GIS (ArcGIS version 10.3 ESRI, Redlands, CA, USA).

2.3.2. Treatment and diagnosis of dogs in sheep farms

2.3.2.1. Dogs treatment and faecal samples collection. In each sheep farm included in the study area, the dogs present were treated and subjected to post-treatment parasitological examination. For each farm, a questionnaire concerning the number of dogs present and if any anthelmintic treatments were used in the previous six months was compiled. The programme involved veterinary practitioners who received the training necessary for the activities and were provided with a kit containing the study protocol, questionnaires, anthelmintic drug, Personal Protective Equipment (PPE), and a sprayer pump with a 5 % solution of sodium hypochlorite and a flamethrower to disinfect the confinement area. In order to facilitate the treatment of dogs and faecal sample collection, the EchinoCage was specifically designed and purpose-built to allow: i) the confinement of dogs for 48 h; ii) the administration of the anthelmintic; iii) the collection of faecal samples and iv) the disinfection of the area, respecting the standards on animal welfare and safety of staff. The cage consisted of eight different panels, one of which being a gate, that formed an octagonal area of 17 square meters. The cage was easy and quick to assemble, suitable for any type of soil and floor (Fig. 2a).

Each studied farm was provided with the EchinoCage where the dogs were confined for 48 h. The treatments were conducted at Day 0, using tablets containing a combination of milbemycin oxime (0.5 mg/kg) and praziquantel (5 mg/kg) (Milbemax®-chewable tablets, Elanco Italia S.p. A). All the faecal samples in the cage (pools) were collected at 24 and 48 h after the treatment (Day 1 and Day 2, respectively). After the 48 h, dogs were released and the area was disinfected with a 5 % solution of sodium hypochlorite or with flame, depending on the type of surface (Fig. 2b). Finally, in order to determine the treatment efficacy, faecal samples were collected 50 days after the treatment (Day 50) in all the sheep farms, using the EchinoCage as described above.

2.3.2.2. Parasitological analysis. Copromicroscopic exams were performed at Day 1 (24 h), Day 2 (48 h) and Day 50 (1200 h) post-treatment. A diagnostic hood (EchinoHood) was specifically designed and purpose-built to guarantee the safety of the laboratory technicians during the preparation of the faecal sample, due to the high zoonotic potential of *E. granulosus* eggs. The EchinoHood (100 × 60 × 60 cm in size) consisted of a steel work bench (similar to a laminar flow hood), a well for storing samples to analyze, an opening in communication with a disposable container for hazardous medical waste, a removable support for Fill-FLOTAC (Cringoli et al., 2017) with 10 holds so it allowed to manipulate simultaneously 10 fresh faecal samples in full safety for the laboratory technician (Fig. 3).

For each farm and at each time of collection (Day 1, Day 2 and Day 50), the faecal samples were pooled, using equal amounts from each individual sample as described by Rinaldi et al. (2015).

In detail, the sample preparation was performed with the Fill-FLOTAC 2 (Cringoli et al., 2017), filling the conic collector with two grams of each pool and adding in the Fill-FLOTAC beaker 18 mL of tap water (dilution ratio = 1:10). The suspension was then thoroughly homogenized and filtered, filling a conical tube with 6 mL of suspension. After centrifugation of the tubes (for 3 min at 1500 rpm at room temperature), the samples were analyzed with the FLOTAC double technique (analytical sensitivity = 2 eggs per gram of faeces, EPG) (Cringoli et al., 2010) using a zinc sulphate flotation solution (specific gravity, s.g. = 1.35). Each FLOTAC was disinfected through a series of washes with a 5 % sodium hypochlorite solution, to inactivate any *E. granulosus* eggs present on its surface, before analysis under the microscope.

After the analysis, the surfaces of the EchinoHood were disinfected using a 5 % sodium hypochlorite solution for 1 h and all the instruments utilized (i.e. conic tubes, Fill-FLOTAC, FLOTAC) were disinfected in a 60 °C water bath, overnight.

The whole process from the preparation to the analysis of the faecal samples as well as the disinfection of the laboratory equipment and devices followed the guidelines recommended by WHO concerning the safety precautions for Echinococcosis/Hydatidosis (WHO/OIE, 2002).

2.3.2.3. Molecular analysis. All the samples resulted positive for Taeniidae eggs were analyzed by molecular techniques for species identification. First, each FLOTAC, containing samples resulted positive for Taeniidae eggs, was placed in a water bath at 60 °C for 2 h to inactivate any *E. granulosus* eggs present before the faecal suspensions were used for molecular analysis. Then, the faecal suspensions with eggs were recovered from the FLOTAC chambers and DNA from all samples was extracted with a commercial Kit QIAamp Stool (Qiagen) (Maurelli et al., 2018).

A multiplex PCR was performed to amplify the small subunit of ribosomal RNA (rrnS) using a mix of primers: Cest1, Cest2, Cest3, Cest4 (2 μM), and Cest5 (16 μM), as reported in Trachsel et al. (2007), in a final volume of 50 μl containing 2 μl of template DNA, 0.2 mM of each dNTP (Sigma-Aldrich, Germany), 2.5 mM of $MgCl_2$, and 2 U of FastStart Taq DNA Polymerase (Life Technologies, USA).

The PCR products were detected on a 2 % ethidium bromide-stained low melting agarose gel (BIO-RAD, Spain). Bands were cut from the gel under UV exposure, and the amplified DNAs were purified by the QIAquick Gel Extraction kit (Qiagen, Germany) and sequenced. The sequences, in both forward and reverse directions, were analysed using the Chromas version 2.1 software and compared with rrnS Taeniidae sequences present in GenBank, using BLASTn system and ClustalW.

2.3.2.4. Statistical analysis. Statistical analyses were performed using SPSS® software (version 22.0, IBM Corporation, Armonk, USA). Since the data did not satisfy normality assumptions, non-parametric methods were used. Summary statistics were obtained by cross-tabulations of categorical data and statistically significant differences in the proportions of positive samples between Day 1 and Day 2 post-treatment were determined using the McNemar's chi-square tests. The Mann Whitney test was used to assess the statistical significance of the difference between *E. granulosus* EPG at 24 and 48 h. The test results were considered significantly different when P-value was < 0.05.

2.3.3. Slaughterhouses' activities

2.3.3.1. Active and passive surveillance. Active and passive surveillance were conducted on ruminant livestock (sheep, goats, cattle and water buffaloes) in the region. Specifically, an active surveillance was carried out monthly in eight "*sentinel*" abattoirs, selected according to their: (i) capacity (number of animals slaughtered per month) and (ii)

EchinoCamp tasks

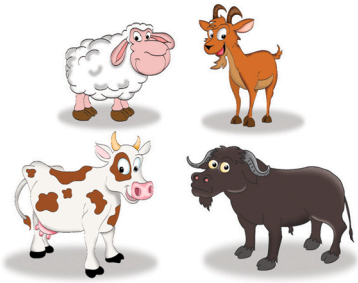
Phase 1		Phase 2
Treatment and diagnosis (24h post-treatment)		Treatment and diagnosis (24 and 48h post-treatment) using the EchinoCage and the EchinoHood, respectively
Active surveillance in sheep at slaughterhouses		Active and passive surveillance in livestock at slaughterhouses Evaluation of the use of ultrasound (US) techniques in detecting hydatid cyst in sheep
Surveillance in humans through HDRs analysis		Surveillance in humans through HDRs analysis
		Food Chain Monitoring through the analysis of the vegetables collected from the sheep farms surveyed.
Outreach activities (information, dissemination and health education) to dogs' owners, farmers, school-age children and physicians		Outreach activities (information, dissemination and health education) to dogs' owners, farmers, school-age children and physicians
		Development of a Virtual Reality (VR) platform to explain in an easy and amusing way the echinococcosis life-cycle and prevention measures.

Fig. 1. EchinoCamp tasks of each phase divided according to the different actions.

Fig. 2. EchinoCage. (a) Confinement of the dogs by using the EchinoCage; (b) Disinfection of the area.

Fig. 3. EchinoHood. (a) Laboratory technician while working under the EchinoHood; (b) Aliquotation of 10 fresh faecal samples using the Fill-FLOTAC collectors; (c) Removal of the support with the Fill-FLOTAC after aliquoting of the faecal samples; (d) 10 fresh faecal samples ready to be processed.

geographical location (to be representative of the whole region).

For each animal slaughtered, CE detection was performed by visual inspection, palpation and incision. During the slaughterhouses' activities a training on organ inspection for the CE detection was offered to staff working in abattoirs by the veterinary practitioners enrolled in the study.

In addition, passive surveillance was performed annually by collecting data regarding the farms with CE positive animals detected at slaughterhouse, using the Italian Veterinarian National Database (BDN) of the Italian Ministry of Health (https://www.vetinfo.it).

2.3.3.2. Assessment of the ultrasound (US) technique for hepatic CE detection. Before being slaughtered, one hundred-seventy-two (172) female sheep of several breeds were subjected to ultrasound (US) examination. A complete liver US examination (cUS) was performed with a Mylab® Alpha device (Esaote SPA, IT) using a microconvex multifrequency transducer (6−10 MHz) as described by Borriello et al. (2021). To assess the sensitivity and specificity of cUS for the detection of hepatic CE, the results of the US were compared with the results of parasitological *post-mortem* examination.

2.3.4. Hospital discharge records (HDRs) analysis

The hospital discharge records (HDRs) data regarding the period from 2016 to 2018, in anonymous form and free of personal information, were provided by the Italian Department of Health. The HDRs contained an anonymous individual code for tracking patient's hospital admissions, discharges and readmissions. Each anonymous individual code identified one patient. Specifically, the database included: patient's admission and discharge dates, gender, age, domicile code, nationality of origin, educational level (if applicable), type of hospitalization (ordinary hospitalization or day hospital).

2.3.5. Vegetable sample analysis

2.3.5.1. Comparison among five protocols for the detection of Taeniidae eggs in vegetables. Different protocols using the following five different washing solutions were compared for isolation and concentration of Taeniidae eggs from vegetables: i) deionized water, ii) 0.1 % Tween 20 (Merck, Germany), iii) 0.1 % Sodium Dodecyl Sulphate (SDS) (Merck, Germany) (Macarisin et al., 2010), iv) 0.1 % Alconox (Merck, Germany) (Frey et al., 2019), v) 1 M Glycine (Merck, Germany) (Frey et al., 2019). In order to standardize the technique, 'ready to eat' salads were purchased from industrial brands and divided in five aliquots of 25 g each. Each aliquot was experimentally contaminated with 600 Taeniidae's eggs (previously isolated from positive faecal samples using the recovery method described in Maurelli et al., 2018) and mixed with 200 mL of washing solution for 3 min. The solution was transferred in 50 mL centrifuge tubes (no. = 4) and subsequently left to sediment for 60 min at room temperature. The supernatant liquid was discarded and the pellets from all tubes were transferred into a 15 mL centrifuge tube that was, then, centrifuged at 1200 rpm for 3 min. The supernatant was discarded and the pellet was analyzed using the FLOTAC technique by adding 6 mL of a zinc sulphate flotation solution (s.g. = 1.35) according to Maurelli et al. (2018).

2.3.5.2. Analysis of vegetable samples in positive farms. In the sheep farms where dogs resulted positive for Taeniidae eggs, vegetable samples were collected and pooled in one sample per farm. Each farm-fresh salad was frozen at −80 °C (WHO/OIE, 2002) and analyzed as described above using glycine 1 M as washing liquid (see results section). Finally, from each sample resulted positive for Taeniidae, the floated suspension was collected and used for molecular analysis as described by Maurelli et al. (2018).

2.3.6. Outreach activities

Dissemination meetings for dogs' owners, farmers and school-aged children as well as physicians, were organized throughout the duration of the programme. The objectives of these activities were focused mainly on providing key information regarding: (i) the parasite life-cycle; (ii) actions to prevent infection in dogs; (iii) actions to avoid infection of the human population.

Educational materials (brochures, posters, gadgets and videos) were designed to support the activities of the health education.

Furthermore, an innovative dissemination tool based on gamification was introduced, namely the Oculus Rift headset, which allows the reproduction of virtual reality VR, 360° images and videos. Specifically, a 360° video was developed, in collaboration with the Education Office of the Campania region, to explain in an easy and amusing way the echinococcosis life-cycle and prevention measures with the final goal of further improving ability of the programme to fulfill learning outcomes.

3. Results

3.1. EchinoCamp – Phase 1

A total of 485 sheep farms were surveyed during the first phase. The main critical point encountered during this phase was related to the treatment of all dogs present in each farm. This was mainly due to the difficulty to confine dogs for 24−48 h in one area, respecting animal welfare standards and avoiding environmental contamination by *E. granulosus* proglottids/eggs eliminated. Therefore, the number of dogs treated with praziquantel (no. = 549) was lower than the actual number of dogs present in the farms (no. = 2182), i.e. an average of 1.1 dogs

treated *versus* 4.5 dogs present in each farm.

Table 1 reports the results obtained during the activities of EchinoCamp-Phase 1 divided by investigated hosts (dogs, sheep and humans).

3.2. EchinoCamp – Phase 2

3.2.1. Treatment and diagnosis of dogs in sheep farms

A total of 1514 dogs were recorded in all the sheep farms with an average of 5.1 dogs per farm. None of them had been subjected to anthelmintic treatment in the previous six months. The number of dogs treated and analyzed during the programme was 1336 (88.2 %) with a mean of 4.5 dogs per farm.

A total of 891 pools of faecal samples was examined, corresponding to the sum of pools of faecal samples collected in each sheep farm and at each time point (Day 1, Day 2 and Day 50). Taeniidae eggs were found at both 24 and 48 h post-treatment (Day 1 and Day 2), whereas none was found at Day 50 (Table 2). Taeniidae eggs were found in 57 sheep farms (19.2 %; 95 % Confidence Interval [CI] = 15.0–24.2).

Molecular analysis showed that 1.7 % (5/297) (95 %CI = 0.6–4.1) farms were positive for *E. granulosus*, 14.5 % (43/297) (95 %CI = 10.8–19.1) for *Taenia hydatigena* and 4.0 % (12/297) (95 %CI = 2.2–7.1) for *T. pisiformis*. Co-infections between different species of Taeniidae were found in three sheep farms, specifically, in one *E. granulosus* and *T. hydatigena* and in two *T. hydatigena* and *T. pisiformis*.

No statistically significant differences were found in the proportions of positive samples according the time of post-treatment (p = 0.345) and between EPG at 24 and 48 h (p = 0.4199).

3.2.2. Slaughterhouses' activities

All the data obtained during the active surveillance were transmitted to the BDN. Table 3 shows the results obtained during the slaughterhouses' activities with the prevalence of animals and farms according to the host species investigated (sheep, goats, cattle and water buffaloes). The results both from the active and passive surveillance showed a marked downward trend of CE prevalence in all the animal species investigated over the years. Specifically, the presence of CE decreased by a total of 30 % in sheep, 27 % in goats, 25 % in water buffaloes and 16 % in cattle.

The cUS technique showed a high value of sensitivity (91 %) and specificity (84 %) compared to *post-mortem* examination. Specifically, of the 172 animals examined, 80 were positive for hepatic CE at *post-mortem* examination and the cUS was able to detect 73 true positive animals (73/80 = 91.2 %; 95 %CI = 82.2–96.1) (Borriello et al., 2021).

3.2.3. Hospital discharge records (HDRs) analysis

A total of 168 HDRs with diagnosis of CE were registered between 2016 and 2018 in the study area, showing an incidence of human CE hospitalizations in Campania region of approximately 0.96/100,000 inhabitants. Most of the HDRs referred to ordinary hospitalizations (81 %), whilst the others to day hospital (19 %). Most patients with CE aged between 60 and 80 years and had a low-level of instruction, whilst no difference was found regarding their sex. The HDR analysis showed a greater spread of the disease in agro-pastoral areas. Despite the HDRs belonged to resident patients in the study area, some of them had foreign origins the common nationality being from Romania, Morocco and Bulgaria.

Table 2
Copromicroscopic examination of pooled samples of dogs: prevalence and egg per gram of faeces (EPG) values (mean, minimum and maximum) of Taeniidae eggs according to the time of post-treatment.

Time of post-treatment	No. pools examined	No. pools positive for Taeniidae eggs	Prevalence (%)(95 %CI)	EPG	
				Mean	Min-Max
Day 1	297	36	12.1 (8.7–16.5)	40.4	2–3740
Day 2	297	46	15.5 (11.7–20.2)	9.9	2–494
Day 50	297	0	0.0 (0.0–1.6)	0.0	0

3.2.4. Analysis of vegetable samples

The most efficient washing solution to concentrate the Taeniidae eggs resulted glycine 1 M which gave the best results in terms of egg recovery rate and easy to use (Table 4).

A total of 57 vegetable samples were analysed, of these three were positive for Taeniidae eggs (5.3 %; 95 %CI = 1.4–15.5) with FLOTAC. Molecular analysis showed that one sample (one sheep farm) was positive for *E. granulosus* (1.7 %; 95 %CI = 0.1–10.6), whilst two samples (two sheep farms) resulted positive for *T. hydatigena* (3.5 %; 95 %CI = 0.6–13.2).

3.2.5. Outreach activities

A total of 200,000 dissemination materials including brochures, posters and gadgets (e.g. hats, pens) about the disease with cartoon pictures suitable for both children and adults were produced and distributed to dogs' owners, farmers and school-aged children. Furthermore, two types of posters, one with all the morphological aspects of the hydatid cysts in the human (no. = 500) and another with the WHO/IWGE classification of ultrasound images of cysts (no. = 500) were produced and distributed to physicians, particularly, general practitioners, pediatricians and radiologists. The dissemination material (in Italian) is available on the website specifically developed for the EchinoCamp project (https://www.echinococco.it/materiale.xhtml).

A total of 80 dissemination meetings were organized with primary and middle school students, farmers, physicians and veterinarians to explain the different aspects of echinococcosis, as the disease, the life-cycle, and the best preventive measures. During these meetings, the Oculus Rift headset (Fig. 4) was introduced for the first time in such kind of programmes. Thanks to this tool the participants, especially children, were immersed in a VR where they could learn by the gamification process (https://www.parassitologia.unina.it/news/virtual-tour-echinococcosiidatidosi_6006.xhtml).

4. Discussion

The control of CE is a still a global concern and to date, only five islands, i.e. Iceland, New Zealand, Tasmania, Northern Cyprus and the Falkland Islands (Las Malvinas), have declared elimination status for *Echinococcus* spp. (Craig et al., 2017). By contrast, other control programmes (e.g. Argentina, Uruguay, La Roja-Spain, Sardinia-Italy) have not had the same level of success. Most of programmes are based on the integration of different tasks as control strategies, e.g. education campaigns, anthelmintic treatment of dogs and sanitary inspection in slaughterhouses (Craig et al., 2017), considering that interruption of

Table 1
Data of prevalence and incidence of *E. granulosus* in dogs, sheep and humans, obtained from the EchinoCamp – Phase 1.

Dogs		Sheep				Humans	
No. dogs Positive/ Analysed	Prevalence (%) (95 %CI)	No. sheep Positive/ Analysed	Prevalence (%) (95 %CI)	No. farms Positive/ Analysed	Prevalence (%) (95 %CI)	No. HDRs	Incidence/100,000 inhab
33/549	6 (4.2–8.4)	683/911	75 (72.0–77.7)	375/463	81 (77.1–84.1)	444	1.19

Table 3
Overall prevalence of cystic echinococcosis in individual animals and farms (sheep, goats, cattle and water buffaloes) obtained from slaughterhouses' activities.

Host species	No. animals analyzed	No. animals positive	Prevalence (%)(95 %CI)	No. farms analyzed	No. farms positive	Prevalence (%)(95 %CI)
Sheep	417	219	52.5 (47.6–57.4)	69	35	50.7 (38.5–62.9)
Goats	125	49	39.2 (30.7–48.4)	29	13	44.8 (27.0–64.0)
Cattle	383	24	6.3 (4.1–9.3)	123	8	6.5 (3.1–12.8)
Water buffaloes	557	31	5.6 (3.9–7.9)	181	10	5.5 (2.8–10.2)

Table 4
Results of the comparison of five washing liquids for the recovery of the Taeniidae eggs from vegetable samples.

No.	Washing solution	Recovery rate	Easy to use
i	Deionized water	2 %	Not good separation between pellet and supernatant
ii	Tween 20	1 %	Too much foam
iii	Sodium dodecyl sulphate	1 %	Too much foam
iv	Alconox	5 %	Too much foam
v	Glycine	98 %	Good separation between pellet and supernatant

Fig. 4. Participant interacting with the virtual reality (VR), via an Oculus Rift headset and motion controllers. The monitor on the top shows live video on echinococcosis.

E. granulosus transmission requires animal-centered interventions in the form of long-lasting integrated control programs (Tamarozzi et al., 2020). Based on this awareness, EchinoCamp, an integrated approach to control CE in the endemic Campania region of southern Italy, has been implemented since 2010 introducing new strategies and tools as described below.

It is well known that carrying out a canine echinococcosis prevention and control programme can significantly decrease the *Echinococcus* spp. prevalence (Larrieu et al., 2019a). However, deworming activities are usually performed to dogs tied to a chain or to unrestrained dogs, in the last case defecation places are pinpointed by the owners. As a consequence, the number of treated dogs might be lower than the total dog population estimated (Šarkūnas et al., 2019) as we observed in the first phase of EchinoCamp. Therefore, the second phase of our programme was based on the use of the purpose-built EchinoCage that made possible to treat and analyze almost all the dogs present on the selected sheep farms. The confinement of the dogs is a crucial phase aimed at enrolling for treatment the largest number of dogs in the farms. Furthermore, the EchinoCage has proved to be an excellent tool for the collection of faecal samples, the elimination of the Taeniidae eggs being both at 24 and 48 h post-treatment, as previously shown by Guo et al. (2014). This result suggests that to control the transmission of echinococcosis it is advisable to properly dispose of the dog faeces for 48 h after the treatment to avoid environment contamination by Taeniidae eggs. The EchinoCage also allowed the removal of *E. granulosus* eggs from the environment, thereby preventing transmission.

Laboratory standard operating procedures (SOPs) have also been successfully developed, harmonized and applied during EchinoCamp to improve the diagnosis while maintaining the safety of the laboratory staff. Specifically, the use of a purpose-built EchinoHood and standardized disinfection procedures has allowed a safe handling and processing for dog's faecal samples in the EchinoCamp laboratories. Furthermore, the use of closed devices for copromicroscopy, i.e. Fill-FLOTAC (Cringoli et al., 2017) and FLOTAC (Cringoli et al., 2010), contributed to avoid the biohazard risks for lab technicians.

The findings of the present study confirmed, also, that praziquantel was highly effective against Taeniidae, in fact no eggs were detected in any of the dog faecal samples, 50 days after deworming. Stray dogs were not included in the control programme and this is a limitation of the study, because of their important role in maintaining the transmission of echinococcosis (Cringoli et al., 2007). Future use of unmanned aerial vehicles (UAVs) for the distribution of baits laced with praziquantel could be a useful strategy for deworming stray dogs as shown by Yu et al. (2017). A control strategy consisting a combination of anthelmintic dog treatment and livestock vaccination offers encouraging prospects for prevention and control (Larrieu et al., 2019b), however the European manufacture and registration of the recombinant EG95 vaccine is still an issue.

From the results at the slaughterhouses, the annual trends for ruminant livestock showed a decrease of CE prevalence during the years, up to 30 % in sheep, demonstrating the effectiveness of the EchinoCamp control programme. Monitoring the CE in livestock at slaughterhouse is, indeed, considered a feasible and economic way to assess and monitor the effect of CE control programme (Yang et al., 2015). Tracking the infected animals that arrive at abattoirs and identifying their origin is of fundamental importance to identify the risk area (Irabedra et al., 2016) and apply appropriate control measures. To date, the diagnosis of CE in livestock species is still performed only at *post-mortem* inspection (Craig et al., 2015). However, early diagnosis of CE in intermediate hosts may provide an excellent prospect for improved control programs. Serological tests have long been considered a potential tool for surveillance in intermediate hosts, but several studies demonstrated cross-reactivity with other taeniid species (Craig et al., 2017). Encouraging results have been obtained from the assessment of the cUS technique for the early hepatic detection of CE in sheep in our programme (Borriello et al., 2021) as in other studies (Dore et al., 2014; Hussein and Elrashidy, 2014). The *in vivo* diagnosis of CE in sheep could interrupt the epidemiological chain of transmission and, therefore, prevent dog and human infections. On the other hand, the early diagnosis and treatment of humans have no effect on parasite transmission, since they represent accidental dead-end intermediate hosts and therefore, they do not

biologically contribute to perpetuating the parasite's life cycle. Instead, surveillance in humans is crucial for targeting interventions, as well as to monitor the progress of the control program (Craig et al., 2017; Tamarozzi et al., 2020). In our study, analysis of HDRs was considered as a proxy to assess the impact of CE in residents of the Campania region. The results evidenced that the disease continues to be a public health problem in this area with an incidence of 0.96/100,000 inhabitants, lower than the national incidence (1.4/100,000 inhabitants) (Piseddu et al., 2017). It is important to note that the HDRs do not include cases managed in an outpatient setting or asymptomatic cases, so the data represent only the tip of the iceberg of the real burden of the CE. The results obtained confirmed the association between the disease and the pastoral communities as reported in the literature (Dakkak, 2010; Deplazes et al., 2017). Furthermore, in agreement with other studies carried out in Italy, the higher frequency of CE has been reported in patients over 60 years (Piseddu et al., 2017) and Morocco and Romania were the countries of the majority of foreign-born patients analyzed (Tamarozzi et al., 2015).

The findings of our study have also important implications for food safety and emphasize the role of raw vegetables in the echinococcosis epidemiology. The presence of Taeniidae eggs, and in particular of *E. granulosus* eggs, in lettuce samples clearly indicates that consumption of contaminated raw vegetables enhances a risk of contracting CE by humans, as shown previously for *E. multilocularis* (Lass et al., 2015; Federer et al., 2016). This confirms the importance of washing the vegetables properly, before consumption. In our study the glycine 1 M resulted the best method in terms of recovery rate and ease of use to isolate and concentrate the Taeniidae eggs. Furthermore, the results obtained confirmed that the FLOTAC technique is able to detect the parasitic eggs on contaminated vegetables as recently shown by do Nascimento Ramos et al. (2019). However, further investigations are needed to standardize the procedures for different vegetable matrices for epidemiological studies and control programmes.

It is accepted by the scientific community that a good control programme should include an education program (Larrieu et al., 2019a), to convince people to actively participate in preventive actions (i.e. dog dosing, reduction in offal being fed to dogs, improved hygiene). During EchinoCamp the traditional dissemination tools have been further implemented by using VR for the first time. VR is being integrated into many different areas of our lives and its applications are continuously increasing. Excellent results are being obtained using this tool for teaching, learning and training (Izard et al., 2018) and in our programme it has proved to be a good dissemination approach, thanks to its ability to provide a robust toolkit for an informed but non-expert user.

Over eight years, EchinoCamp has resulted in a reduction of the infection rates of dogs, livestock and humans in the Campania region thus demonstrating that integrated multidisciplinary and multi-institution efforts are required for a successful control programme, within a One Health perspective (Wen et al., 2019).

The main outcome of this control programme is the translation of research results into policy (Trevisan et al., 2019). Indeed, based on the achievements obtained during EchinoCamp, the Campania regional authorities have developed a specific regulation undertaken and managed by the Veterinary Regulation Department on which the procedures and tools adopted during the programme must be used regionally to target interventions aimed to further control CE in animals and humans. The above mentioned combined interventions could be an effective, sustainable scheme for CE management, surveillance and control, to be adopted by national and international health authorities to strength the disease surveillance capabilities and reduce the burden of human echinococcosis, as well as to improve health, welfare and productivity of livestock in other endemic Mediterranean areas.

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CRediT authorship contribution statement

G. Cringoli: Supervision, Conceptualization, Methodology, Validation, Resources, Writing - review & editing. **P. Pepe:** Investigation, Methodology, Data curation, Formal analysis, Writing - original draft. **A. Bosco:** Investigation, Methodology, Data curation, Formal analysis, Writing - original draft. **M.P. Maurelli:** Investigation, Methodology, Data curation, Formal analysis, Writing - original draft. **L. Baldi:** Conceptualization, Methodology, Validation, Resources, Writing - original draft. **P. Ciaramella:** Investigation, Formal analysis, Resources, Writing - original draft. **V. Musella:** Data curation, Formal analysis. **M. L. Buonanno:** Investigation, Methodology, Data curation. **F. Capuano:** Investigation, Formal analysis, Resources, Writing - original draft. **F. Corrado:** Investigation, Methodology, Data curation, Writing - original draft. **D. Ianniello:** Investigation, Methodology, Data curation. **L.C. Alves:** Investigation, Methodology, Formal analysis, Writing - original draft. **P. Sarnelli:** Supervision, Project administration, Funding acquisition, Writing - review & editing. **L Rinaldi:** Supervision, Conceptualization, Methodology, Validation, Resources, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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