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A new approach to outbreak management for bovine Cystic Echinococcosis cases in hypo-endemic areas

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ABSTRACT

Cystic Echinococcosis (CE) surveillance in Italy is based on detection of its larval stage (hydatic cysts) at the slaughterhouse. In northern Italy, a hypo-endemic area, local health authorities investigate each individual farm with positive animals to treat their dogs with cestocidal drugs, but this system is time-consuming and poorly effective for bovine farms. The study applied a new approach based on targeted epidemiological surveys in areas with aggregation of bovine CE cases, and compared the outcome with that of two control areas with farms individually investigated.

The presence of territorial cluster of bovine farms with CE cases was investigated for 3 consecutive years (2013–2014–2015) in a high-risk area of Veneto Region (north-eastern Italy), using a spatial scan statistic. Epidemiological investigations, consisting of a questionnaire survey and canine faecal samples collection, were conducted in cluster and control areas. All faecal samples were analyzed for taeniids eggs and positive samples were sequenced and identified.

In total, 99 farms were surveyed and 208 faecal samples were retrieved from dogs. Sixty-two farms (42 bovine and 20 sheep) were investigated in cluster areas and 37 farms (33 bovine and 4 sheep) in control areas. Based on the results of the cestode egg isolation procedure, 14 animals (6.7%) were positive to taeniid eggs. For molecular analysis, two dogs resulted positive to *Echinococcus granulosus*, and seven to *Taenia hydatigena*. Twelve positive dogs were found in targeted survey areas and ten of these dogs were shepherd dogs, belonging to transhumant sheep flocks known to pass in cluster areas.

The new approach demonstrated to successfully identify the probable source of infection of CE positive bovines. Most of positive dogs belonged to transhumant flocks, underlining the importance to include shepherd dogs in the surveillance system for CE.

1. Introduction

Cystic Echinococcosis (CE) is an important zoonotic parasitic disease caused by *Echinococcus granulosus* sensu lato, which represents a serious chronic and disabler disease in humans (Budke et al., 2017). Cattle act as intermediate hosts for *E. granulosus*, as many other domestic and wild mammals, harbouring the larval stage (hydatid cyst). The disease is widespread in southern and south-eastern Europe, particularly in areas where sheep herding still represents an important contribution to the local economy, such as in the Balkan, Italian and Iberian peninsulas (Carmena and Cardona, 2013; Tamarozzi et al., 2018). Central and southern Italy (including major Islands) are characterized by a medium to highly endemic status, highlighted by prevalence values ranging from 5% to 80% in sheep and from 3 to 70% in cattle (Garippa and Manfredi, 2009). On the contrary, CE is rarely reported in Northern Italy, where prevalence in cattle populations is generally below 1% (Guazzetti et al., 2006; Manfredi et al., 2011; Cassini et al., 2014). However, rare autochthonous cases of dogs with *E. granulosus* were recently reported in this part of the country (Manfredi et al., 2011) and specifically in Veneto Region (Cassini et al., 2014). Consequently, increased attention has been attributed to the emerging of CE in this hypo-endemic area, where tailored surveillance system needs to be adopted.

During the considered period (2013–2015), Veneto Region was

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characterized by an important presence of about 850,000 bovines, whereas sheep population accounted for around 27,000 head (http://agri.istat.it/). Very few semi-intensive sheep farms are found in the Region, mostly dairy farms. Besides, about 60 transhumant sheep flocks with an average of some hundreds of adult animals each move continuously in the territory of north-eastern Italy for the whole year. Most of them are passing through the agricultural fields and pasture areas of bovine farms of the Region (Nori and De Marchi, 2015). As a consequence, bovine population may act as sentinel for *E. granulosus* environmental contamination (Rinaldi et al., 2008) and the use of bovine CE cases for retrospective analysis appears appropriate in consideration of the high number of slaughtered heads (about 30,000 dairy cattle per year) and of the high reliability of the national bovine identification database (BDN).

According to European Directive No. 2003/99/EC, active monitoring for echinococcosis shall take place at the most appropriate stage of the food chain in all Member States. According to the Regulation (EC) No 854/2004 (Annex I, Section II, Chapter I, art. 2b) the official veterinarian at the slaughterhouse, carries out a visual inspection of offal accompanied by standardized incision. This standard inspection is appropriate to detect CE. In the case of positive animals, these are notified to the local competent authority that conducts an epidemiological survey in the farm of origin of the positive animal and treats the supposed source of the infection (the farm dog/dogs) using a cestocidal drug. Therefore, according to the this approach, each single case of bovine CE is considered as an outbreak, based on the assumption that the disease should not be present in the territory and therefore positive cases are not expected. In fact, as per the WHO definition, “a disease outbreak is the occurrence of disease cases in excess of normal expectancy”. Nevertheless, this approach appears time-consuming, since the veterinary service is supposed to individually investigate each single bovine farm with positive animal/s. At present, in Veneto Region about one hundred CE bovine cases are reported each year (Cassini et al., 2014). Besides, the effectiveness of the approach is also questionable, since the source of the infection is not clearly demonstrated (faecal samples from bovine farm dogs are seldom analyzed to demonstrate the presence of taeniid eggs) and therefore the treatment of the farm dog/s may be useless. According to what reported by the Regional Laboratory in charge for animal health surveillance (Istituto Zooprofilattico Sperimentale delle Venezie - https://www.izsvenezie.it), none dog belonging to bovine farm has been identified as carrier of *E. granulosus* in the Region so far (unpublished data).

This study experimented a new procedure for an effective management of CE cases from bovine farms, investigating the spatial aspects of CE to identify areas with excess of cases, where to conduct targeted epidemiological investigations. The new approach proposed in this study is shifting the ‘outbreak’ concept from the management of each single case of bovine CE to a territorial approach, where an in-depth survey of the different possible sources of infection, including shepherd dogs, is conducted only in areas with significant aggregation of CE cases, in a defined time-frame. The aim of the study is to investigate the feasibility and the effectiveness of the new proposed approach in identifying positive dogs and to compare it with two control areas where positive farms were individually investigated.

2. Materials and methods

2.1. Retrospective and spatial analysis

Only the Health Units (Azienda ULSS) of Veneto Region considered at major risk on the basis of the results of a previous research (Cassini et al., 2014) were included in the spatial analysis. The study area corresponded to the whole Vicenza Province, the Northern part of Verona and Padova Provinces, and the western part of Treviso Province (Fig. 1). Analyses were conducted on a yearly basis, from 2013 to 2015.

National and Regional computerized databases for bovine, as per Regulation (EC) No 1760/2000, were used to track movements of all identified positive cattle recorded at slaughterhouse. Only positive cases for which it was possible to certainly identify the farm where the infection was acquired (i.e. individuals spending all their life in the same farm, or spending $<$ 4 weeks in other farms at the beginning or at the end of their life) were considered in the spatial analysis.

The defined time-frame for the spatial analyses was set in 12 months. The spatial aggregation of bovines positive to CE in the study area was explored through a purely spatial scan statistic (Kulldorff, 1997), using the Bernoulli probability model, as previously described (Cassini et al., 2014). The years 2013, 2014 and 2015 were considered separately. The size of the spatial scanning window was set to include up to 50% of the total population of cattle farms active in each considered year. A cluster of positive farms was considered significant when the *P*-value was ≤ 0.05.

Data elaboration was performed using SaTScan™ version 9.1.1 (http://www.satscan.org/), while the output was visualized in ESRI™ ArcMap® 10.1 (http://www.esri.com/).

2.2. Epidemiological investigation

From June 2012 to February 2017 targeted epidemiological surveys were conducted in the areas identified as clusters of bovine CE, during a previous research (Cassini et al., 2014) and in the present study. All available bovine positive farms found in the cluster area were visited during the surveys. Also few negative farms were included, because considered ‘at risk’ by the local veterinarians, on the basis of a history of previous reports of bovine CE cases. Neighbouring semi-intensive sheep farms and transhumant sheep flocks known to pass through the area were also investigated, whenever possible. The targeted epidemiological investigation in the farms found in the clusters was associated and compared with the investigation of positive bovine farms found in two control areas (VR2 and TV), during a similar period (May 2013–February 2017). These areas were selected because of a history of medium number of positive cases (Fig. 1) and corresponded to the territories of one Health Unit in Verona Province (VR2) and one Health Unit in Treviso Province (TV). In these Units, veterinary personnel were requested to conduct an interview and to collect canine faecal samples from positive farms, to be analyzed for the presence of taeniid eggs, in addition to the usually performed survey activity (dog treatment). In one control area (TV) also the transhumant flocks roaming for many weeks in the area were included in the investigation.

A structured interview, based on two questionnaire formats (bovine and sheep farms), was conducted with each farmer to collect information on attitudes that can facilitate the introduction of *E. granulosus* eggs in the farm: type of feed used for animals (green grass, mais silage, hay); use of pasture (yes/no); number of owned dogs; presence of stray dogs in the farm (yes/no); dog feeding habits (pet foods, home leftovers); dog anthelmintic treatments (n° treatments/year, type of used drug/s). The results of the questionnaire survey were analyzed by a simple descriptive statistics.

2.3. Sampling and laboratory analyses

Individual faecal samples were retrieved from all available dogs belonging to the farms. Dog owners were provided with containers for faecal samples and advised to supervise their dogs and to collect their faeces the evening before the survey or the morning of the same day. In few cases, samples were collected through rectal inspection. Safety measures were clearly explained to owners and operators.

All canine faecal samples were frozen at $-80\,^{\circ}$C for at least 72 h to ensure any viable egg was killed prior to examination. Two grams for each sample were thoroughly mixed with 8 ml of tap water prior to centrifugation $1600 \times g$ for 10 min, and submitted to the egg isolation procedure described by Davidson et al. (2009). The presence of taeniid eggs was detected by means of an inverted microscope in a 10 ml closed

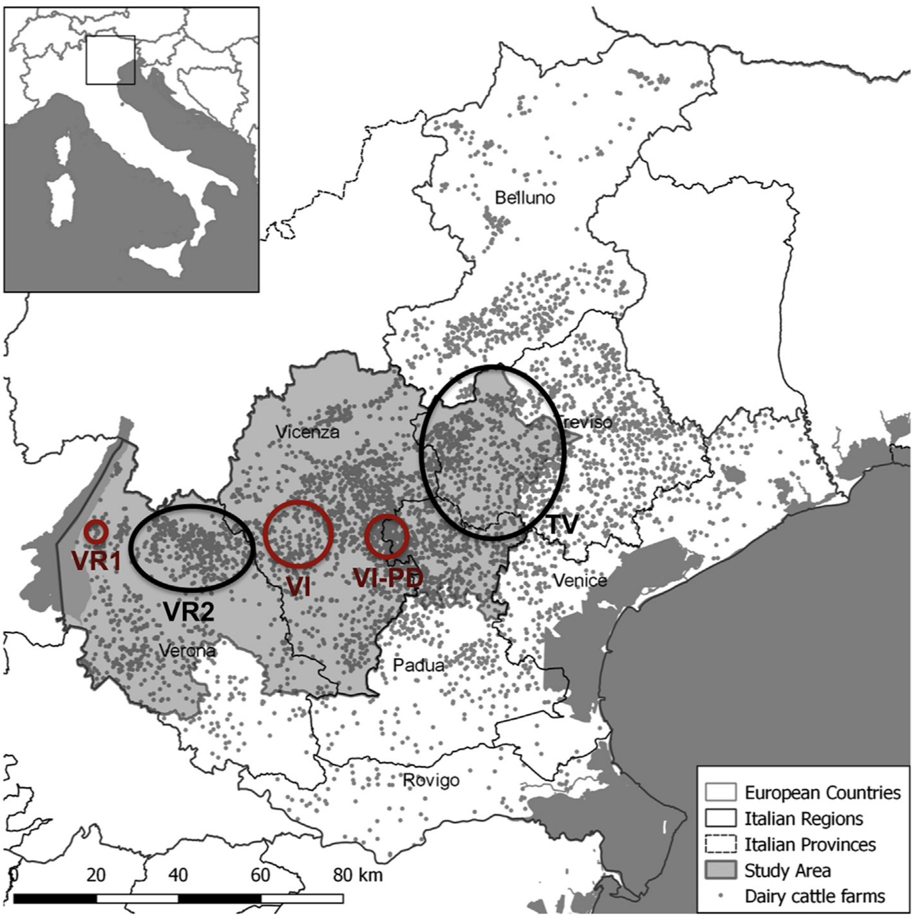

Fig. 1. Map of study area. The area considered in the spatial analysis is in light grey and dark grey dots represent dairy cattle farms. The three areas (VR1, VI-PD, VI) investigated through targeted epidemiological surveys are highlighted by a red circle, whereas control areas (VR2, TV) by a black circle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tube with flattened side, where the liquid obtained at the end of the isolation procedure was carefully flushed. Only positive samples were investigated by a multiplex PCR (Trachsel et al., 2007), after further sedimentation process for 30 min, and aspiration of excess fluid from the tube, in order to ensure a final volume of approximately 0.5 ml. *Echinococcus granulosus* positive samples were confirmed also using a different PCR, targeting COX-I gene (Bart et al., 2006). Amplicons were sequenced and obtained sequences were aligned using BLAST software with those available in GenBank™ database. The results of PCRs were confirmed by the National Reference Centre for *Echinococcus granulosus* (CeNRE, IZS Sardinia).

3. Results

3.1. Cluster identification (2013–2015)

Only dairy cattle were identified as autochthonous cases, and, therefore, only dairy farms were considered as control in the spatial analysis. A total of 25 farms with at least one CE-positive bovine were identified in 2013 in the study area, out of a total of 3914 farms included in the analysis (dairy farms active during the considered year). As of 2014, 33 farms tested positive to CE, and population of reference was represented by 3800 farms. Finally, in 2015, 41 positive farms were compared with a reference population of 3710 farms.

Spatial analysis identified only one significant cluster in 2014 ($p = .001$). The geographical location and the characteristics of the cluster (coded 2014-1) are shown in Fig. 2 and reported in Table 1, respectively.

3.2. Epidemiological investigation

The number of farms and dogs considered in targeted epidemiological surveys (and characteristics of corresponding clusters) and in control areas are reported in Table 1.

The questionnaires were submitted to 99 herders (75 bovine and 24 sheep farms) and their answers highlighted the percentage of farmers adopting attitudes concerned with farm animals management, which can facilitate the spread of the disease and its introduction in the farm.

Concerning bovine farms, only 54% was using pasture, but nearly all (99%) were feeding animals with hay, either self-produced in the agriculture fields around the housing system, or purchased from other areas in North-eastern Italy. Among sheep farms, 18 (75%) were traditional transhumant flocks and 6 (25%) semi-intensive farms. All sheep farms were keeping animals at pasture for the whole year and moving throughout the entire Regional territory in transhumant flocks or locally in semi-intensive sheep farms, where animals are moved to fenced or unfenced pastures during some period of the day.

Owned dogs were present in 95% of bovine farms, with an average of 2.3 dogs per farm. On the contrary, all sheepherders reported to have one or more dogs, with an overall average of 4.3 dogs per farm. The age of examined dogs ranged from 6 months to 20 years, with an average of 5.2 years.

Few herders (11%) declared to feed their dogs only with pet food, 11% with home left-overs, whereas most herders (78%) used both. In 86% of the bovine farms, dogs had free access to the barn, and the presence of stray or free-ranging dogs was reported by 73% of interviewed farmers.

Among bovine farm dogs, 69% were not effectively treated against taeniids (no treatment, deworming only during weaning, rare treatments with drugs without cestodicidal agents), whereas a minority was

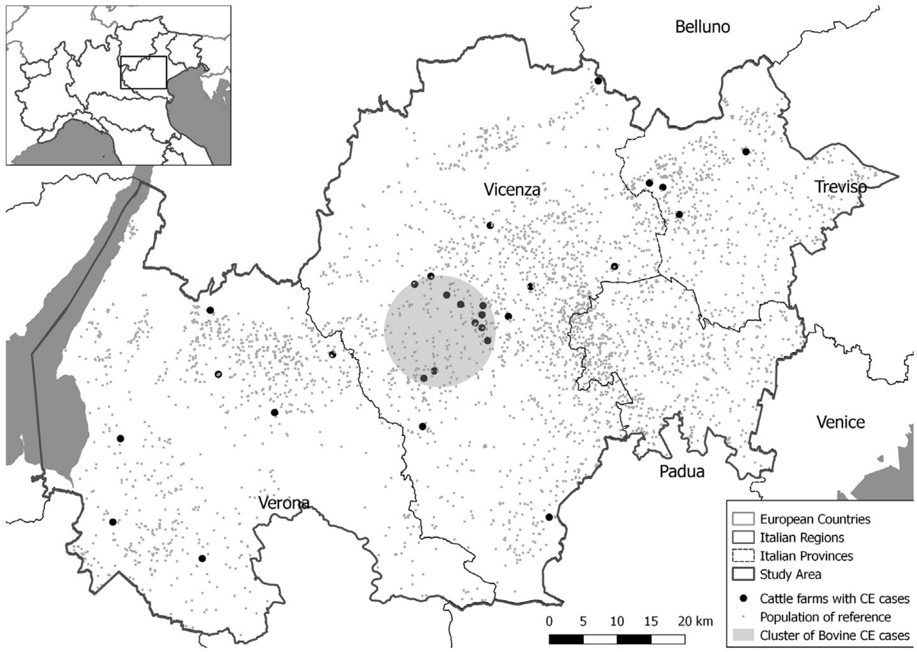

Fig. 2. Geographical localization of the cluster (2014-1) of bovine CE identified in 2014.

treated with effective drugs with unknown periodicity (21%), one time per year (1%) or two or more times per year (9%). On the contrary, 32% of sheep farm dogs were not effectively treated, and 15%, 5% and 48% were treated with unknown periodicity, one time per year and two or more times per year, respectively.

3.3. Parasitological survey

The isolation procedure was performed for all 208 collected samples and allowed the detection of taeniid eggs in 6.7% (14/208; C.I.: 3.3–10.1%) of sampled dogs. The provenance area, the dog typology and the most likely molecular identification of positive samples are reported in Table 2. Taenids eggs were found in twelve dogs in targeted surveys and in only two in control areas. More interestingly, nearly all dogs positive to *T. hydatigena* and *E. granulosus* were shepherd dogs, and the only one classified as bovine farm dog was actually reported by the farmer as purchased from the owner of a transhumant flock (where it lived up to 3 months). At least one dog harbouring either *T. hydatigena* or *E. granulosus* was found in each of the three targeted epidemiological surveys. Unfortunately, it was not possible to perform BLAST analysis for six samples, due to the poor quality of the obtained sequences.

4. Discussion

The method proposed for the management of CE bovine cases demonstrated to be effective. In all targeted epidemiological surveys at least one dog positive to *E. granulosus* or to *T. hydatigena* was found, suggesting that this territorial approach can identify the potential origin of the infestation in the investigated area. On the contrary, the routine surveillance system of the two control areas allowed for the identification of only two dogs positive to taeniid eggs. The retrospective analysis aim was not to identify exactly the single dog at the origin of the eggs shedding that caused the bovine infestation, but to find out the farm with dogs at risk. In these farms, which are found in or are known to regularly pass through the cluster area, dogs are managed in a way that makes possible their infestation with CE: they are free to move in the territory and used to occasionally eat sheep offal. Dogs from sheep farms and in particular from traditional transhumant flocks are more at risk for CE and their inclusion in the targeted surveys was fundamental.

The study identified two dogs positive to *E. granulosus* and seven dogs positive to *T. hydatigena*. This latter parasite does not have the zoonotic importance of *E. granulosus*, but it shares the same life cycle and it is therefore an indicator of a dog with feeding habits at risk for CE infestation (consumption of raw sheep offal). Besides, taeniid eggs isolated from five faecal samples were not identified at species level, but only included among Taenidea group, according to multiplex-PCR results. Considering that all isolates ($n = 7$), for which it was possible to perform an high quality sequencing, were identified as *T. hydatigena*, it is probable that most of these five dogs were harbouring the same parasite. As a consequence, the overall prevalence of sheep-related tapeworms can be estimated equal to 6.7% among farm dogs in Veneto Region. If we refer only to shepherd dogs, a more alarming value of 19.6% (10/51) can be reached. These results suggest that also in Northern Italy traditional transhumant sheep rearing plays an important role in maintaining and spreading tapeworm species characterized by a 'dog-sheep' life cycle, similarly to what reported for Italian Regions endemic for CE (Cringoli et al., 2007; Scala et al., 2004) and more generally for all southern Europe (Carmena and Cardona, 2013). Among these species, also *E. granulosus* has been now demonstrated to circulate and to complete its life cycle sparsely in the territory of Veneto Region.

The system for CE surveillance proposed in this study includes an initial step consisting in a retrospective and spatial analysis aimed at the identification of the territories at higher risk. Two of the three targeted areas (VR1 and VI-PD) were identified as clusters in a previous research (Cassini et al., 2014), thanks to a spatial analysis based on 5-years retrospective survey. However, waiting such a long time to promote a preventive action is not acceptable for a surveillance system. As a consequence, in the present study we decided to verify the feasibility of a retrospective and spatial analysis based on a time-frame of one-year, which is an acceptable time for a surveillance system. The approach proved valid in 2014 (one cluster identified), but it missed to detect any significant aggregation both in 2013 and 2015. This result is suggesting that only areas with high contamination of *E. granulosus* eggs can be identified. A different methodological approach can be used to increase the sensitiveness of the spatial analysis, such as the use of a Poisson probability model (Kulldorff, 1997), which considers positivity at individual level and not at farm level. The procedure to perform scan statistics with Poisson model is more complex and time-consuming, and requires detailed information that were not fully available for our

Table 1
Number of farms and dogs investigated during the epidemiological surveys and main characteristics of relative clusters of bovine CE cases.

ID Area	Area	N investigated farms		N tested dogs		cluster characteristics						
		N bovine farms (positive to CE)	N sheep farms (transhumant flocks)	N bovine farm dogs (from positive farms)	N sheep farm dogs (from transhumant flocks)	Cluster ID	N farms included in the cluster	N observed cases (positive farms)	Radius (m)	*P* value	Year/s considered in Spatial analysis	Reference
VR1	Western Verona	12 (7)	3 (1)	21 (14)	10 (3)	1	18	10	1333	< 0.001	2006–2010	Cassini et al., 2014
VI-PD	Eastern Vicenza and Northern Padova	16 (15)	12 (8)	17 (14)	42 (25)	2	198	22	4219	=0.01	2006–2010	Cassini et al., 2014
VI	Central Vicenza	14 (11)	5 (5)	18 (16)	11 (11)	2014–1	212	12	8227	=0.001	2014	Present study
Total (targeted surveys)		42 (33)	20 (14)	56 (44)	63 (39)							
VR2	Eastern Verona	12 (12)	0	17 (17)	0	Control farms (not included in any cluster)						
TV	Treviso	21 (21)	4 (4)	60 (60)	12 (12)							
Total (control areas)		33 (33)	4 (4)	77 (77)	12 (12)							
Overall Total		75 (66)	24 (18)	133 (121)	75 (51)							

population of reference.

Notwithstanding there is evidence that cases of bovine CE are sometime and somewhere clustering in restricted areas, the distribution of the parasite appears widespread in the Region. It is still difficult to understand which are the dissemination routes used by *E. granulosus* eggs to enter into bovine farms. However, since only 54% of farms were keeping animals at pasture for a period of the year, other ways of entrance, such as the use of contaminated hay, has to be hypothesized. Our data suggest that the farm dog of bovine herders has a very limited role, and it is rarely the source of the infestation of the herd. This finding is stressing once again the limited usefulness of treating only the dog/s of the bovine farms with CE cases. An important role in disseminating *E. granulosus* eggs is instead probably played by shepherd dogs that are moving through the whole plane territory of the Region during winter periods, as per the traditional transhumant system (Nori and De Marchi, 2015). The higher level of exposure to infestation with sheep-related tapeworms of shepherd dogs is clearly perceived by their owners. In fact, nearly half of sheepherders (48%) are treating their dogs two or more times per year with cestocidal drugs, compared with a minority (9%) among bovine farmers. However, other dog typologies such as free-ranging, stray and hunting dogs can have primary epidemiological roles in CE diffusion, also in consideration of their feeding habits, movement freedom and limited level of supervision by human beings. Finally, also the importance of wild animals should be taken into consideration. In the study area, wild canids are very rare (few wolfs have been recently observed), whereas wild boar, roe deer and red deer populations are present and locally abundant. Their role as intermediate hosts has been investigated in other areas of Italy (Paoletti et al., 2018), but not in the study area, so far.

5. Conclusion

The study demonstrated the feasibility of a new system for CE surveillance, based on a territorial approach to outbreak management, with targeted epidemiological investigation in areas with significant aggregation of bovine CE cases and inclusion of transhumant flocks known to pass through the same areas. The proposed approach showed to be able to successfully identify dogs positive to *E. granulosus*, either to *T. hydatigena*, which has a similar life cycle and common risk factors for definitive host infestation.

An improvement of the management of data flow of bovine CE cases with centralisation of data analysis, as per the experience of the present research, is strongly recommended. The centralised data collection and the subsequent spatial analysis on a yearly basis have been demonstrated to be effective in identifying cluster of CE cases, but different methodologies in spatial analysis may help to improve the sensitiveness. Besides, a centralised and regular data collection allows for a constant monitoring of prevalence values among bovine resident population, which may act as indicators of CE infection level in the area, as already suggested previously (Rinaldi et al., 2008).

The results of the present research suggest to the Regional health authorities to develop some feasible policy measures to diminish the risk of infestation in shepherd dogs. An improvement in their regular treatment (e.g. treatment 4 times a year, with an effective drug) can be achieved by an active public policy, but the cost-effectiveness of this kind of intervention has to be evaluated. At the same time, the level of parasitism of other dog typologies and their role in disseminating *E. granulosus* eggs have to be further investigated.

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Table 2
Most likely identification of dog positive faecal samples.

Area	ID Area	N investigated dogs	N positive dogs	Dog typology	Most likely identification	GenBank Accession Number*	Similarity (%)
Targeted surveys	VR1	31	5	sheep farm dog	Taenidae		
				sheep farm dog	Taenidae		
				shepherd dog	Taenidae		
				shepherd dog	Taenidae		
				shepherd dog	*E. granulosus*		
	VI-PD	59	6	shepherd dog	*E. granulosus*	KY766891	100%
				shepherd dog	*T. hydatigena*	KU750812	99%
				shepherd dog	*T. hydatigena*	KU750812	100%
				shepherd dog	*T. hydatigena*	KU750812	100%
				shepherd dog	*T. hydatigena*	KU750812	100%
				shepherd dog	*T. hydatigena*	KU750812	100%
	VI	29	1	shepherd dog	*T. hydatigena*	KU750812	99%
Total		119	12				
Control areas	VR2	17	0				
	TV	72	2	bovine farm dog	Taenidae		
				bovine farm dog	*T. hydatigena*	KU750812	100%
Total		89	2				
Overall Total		208	14				

* GenBank Accession Numbers has been reported only when high quality sequences were obtained.

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Conflict of interest statement

We have no conflicts of interest to disclose.

Ethical statement

In our study animals were kept in natural conditions and samples collected according to standard procedures and according to the principles established by The International Guiding Principles for Biomedical Research Involving Animals (2012).

References

Bart, J.M., Morariu, S., Knapp, J., Ilie, M.S., Pitulescu, M., Anghel, A., Cosoroaba, I., Piarroux, R., 2006. Genetic typing of *Echinococcus granulosus* in Romania. Parasitol. Res. 98, 130–137.

Budke, C.M., Casulli, A., Kern, P., Vuitton, D.A., 2017. Cystic and alveolar echinococcosis: successes and continuing challenges. PLoS Negl. Trop. Dis. 11, e0005477.

Carmena, D., Cardona, G.A., 2013. Canine echinococcosis: global epidemiology and genotypic diversity. Acta Trop. 128, 441–460.

Cassini, R., Mulatti, P., Zanardello, C., Simonato, G., Signorini, M., Cazzin, S., Tambalo, P., Cobianchi, M., Pietrobelli, M., Capelli, G., 2014. Retrospective and spatial analysis tools for integrated surveillance of cystic echinococcosis and bovine cysticercosis in hypo-endemic areas. Geospat. Health 8, 509–515.

Cringoli, G., Rinaldi, L., Musella, V., Veneziano, V., Maurelli, M.P., Di Pietro, F., Frisiello, M., Di Pietro, S., 2007. Geo-referencing livestock farms as tool for studying cystic echinococcosis epidemiology in cattle and water buffaloes from southern Italy. Geospat. Health 2, 105–111.

Davidson, R.K., Øines, Ø., Madslien, K., Mathis, A., 2009. Echinococcus multilocularis — adaptation of a worm egg isolation procedure coupled with a multiplex PCR assay to carry out large-scale screening of red foxes (*Vulpes vulpes*) in Norway. Parasitol. Res. 104, 509–514.

Garippa, G., Manfredi, M.T., 2009. Cystic echinococcosis in Europe and in Italy. Vet. Res. Commun. 33 (Suppl. 1), 35–39.

Guazzetti, S., Micagni, G., Ostanello, F., Battelli, G., 2006. Bovine echinococcosis in the province of Reggio Emilia (Italy): an example of integrated analysis of passive surveillance data. Parassitologia 48, 340.

Kulldorff, M., 1997. A spatial scan statistic. Commun. Stat. Theor. Meth. 26, 1481–1496.

Manfredi, M.T., Di Cerbo, A.R., Zanzani, S., Moriggia, A., Fattori, D., Siboni, A., Bonazza, V., Filice, C., Brunetti, E., 2011. Prevalence of echinococcosis in humans, livestock and dogs in northern Italy. Helminthologia 48, 59–66.

Nori, M., De Marchi, V., 2015. Pastorizia, biodiversità e la sfida dell'immigrazione: il caso del Triveneto. Cult. Della Sostenibilità 15, 78–101.

Paoletti, B., Della Salda, L., Di Cesare, A., Iorio, R., Vergara, A., Fava, C., Olivastri, A., Dessì, G., Scala, A., Varcasia, A., 2018. Epidemiological survey on cystic echinococcosis in wild boar from Central Italy. Parasitol. Res. https://doi.org/10.1007/s00436-018-6112-3.

Rinaldi, L., Maurelli, M.P., Veneziano, V., Capuano, F., Perugini, A.G., Cringoli, S., 2008. The role of cattle in the epidemiology of *Echinococcus granulosus* in an endemic area of southern Italy. Parasitol. Res. 103, 175–179.

Scala, A., Varcasia, A., Garippa, G., 2004. Cystic echinococcosis in Sardinia: the current role of sheep. Parassitologia 46, 397–400.

Tamarozzi, F., Akhan, O., Cretu, C.M., Vutova, K., Akinci, D., Chipeva, R., Ciftci, T., Constantin, C.M., Fabiani, M., Golemanov, B., Janta, D., Mihailescu, P., Muhtarov, M., Orsten, S., Petrutescu, M., Pezzotti, P., Popa, A.C., Popa, L.G., Popa, M.I., Velev, V., Siles-Lucas, M., Brunetti, E., Casulli, A., 2018. Prevalence of abdominal cystic echinococcosis in rural Bulgaria, Romania, and Turkey: a cross-sectional, ultrasound-based, population study from the HERACLES project. Lancet Infect. Dis. 18, 769–778.

Trachsel, D., Deplazes, P., Mathis, A., 2007. Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. Parasitology 134, 911–920.