Echinococcus Multilocularis and other Cestodes in Red Foxes (*Vulpes Vulpes*) of Northeast Italy, 2012-2018

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Abstract

Background

Echinococcus multilocularis is a small tapeworm affecting wild and domestic carnivores and voles in a typical prey-predator life cycle. In Italy, a focus of *E. multilocularis* has been in existence since 1997 in the northern Italian Alps, later confirmed in foxes collected from 2001 to 2005. In this study, we report the results of seven years of monitoring on *E. multilocularis* and other cestodes and describe the changes occurred over time and among areas showing different environmental and ecological features (eco-regions).

Methods

Eggs of cestodes were isolated from faeces of 2,872 foxes with a sedimentation/filtration technique. The cestode species was determined through multiplex PCR, targeting and sequencing ND1 and 12S genes.

Results

Overall, 217 (7.55%) samples resulted positive for cestode eggs at coproscopy, with differences of prevalence according to year and sampling area. Eight species of cestodes were identified, with *Taenia crassiceps* (2.6%), *Taenia polyacantha* (2%) and *E. multilocularis* (1%) as the species most represented. The other species, *Mesocestoides litteratus, Taenia krabbei, T. serialis, T. taeniaeformis* and *Dipylidium caninu*m, accounted for less than 1% altogether. *Echinococcus multilocularis* was identified in foxes from two out of six eco-regions, in 30 faecal samples, accounting for 1.4% within the cestode positives at coproscopy. All *E. multilocularis* isolates came from Bolzano province. Prevalence of cestodes, both collectively and for each of the three most represented species (*T. crassiceps, T. polyacantha* and *E. multilocularis*) varied based on the sampling year, and for *E. multilocularis* an apparent increasing trend across the last few years was evidenced.

Conclusions

Our study confirms the presence of a low prevalence focus of *E. multilocularis* in red foxes of northeast Italy. Although this focus seems still spatially limited, given its persistence and apparent increasing prevalence through the years, we recommend that future work deepen the ecological factors allowing persistence of this zoonotic species at a smaller scale. On the same scale, we recommend an informative campaign on how to behave to avoid contamination, targeted at people living in the area, especially hunters, dog owners, forestry workers and other categories at risk.

Background And Aim Of The Study

Echinococcus multilocularis (Cestoda, Cyclophillidea, Taeniidae) is a small tapeworm affecting wild and domestic carnivores and voles in a typical prey-predator life cycle (1). The principal cycle involves the red fox (*Vulpes vulpes*) as definitive host and small rodents (muskrats and voles) as intermediate hosts. Other carnivores such as the Arctic fox (*Vulpes lagopus)*, the wolf (*Canis lupus*), the raccoon dog (*Nyctereutes procyonoides*), the golden jackal (*Canis aureus*), the dog (*Canis lupus f. familiaris*) and the cat (*Felis catus*), may act as definitive hosts (2–5). The eggs excreted with the faeces by the definitive hosts contaminate the environment and represent the infective stage for the intermediate hosts, including humans. Humans are accidental hosts and may acquire the infection by ingesting contaminated water (6) or fresh fruits, vegetables and mushroom (7). In the intermediate hosts, *E. multilocularis* is the causative agent of alveolar echinococcosis, a serious disease that in humans can be fatal if not treated (8). Indeed, beside rabies, *E. multilocularis* represents the major zoonotic agent transmitted from foxes to humans. At the European level, a group of experts ranked *E. multilocularis* first as a priority infection among food-borne pathogens (9). In north – eastern Italy, the red fox is present from sea level up to the tree line; as in the rest of the Peninsula, it is considered to be generally abundant and does not show particular conservation issues (10). Few studies have addressed local populations densities, reporting values ranging from 1.77 ± 1.14 individuals/km^2 in Friuli–Venezia Giulia region (11) to 2.11 ± 0.56 individuals/km^2 (Celva R., personal communication) in Bolzano Province. During the last two decades, the alpine fox population of our study area recovered from a rabies epizootic, that had started in late 2008 and was eradicated by oral fox vaccination in early 2011 (12). Moreover, since 2006 it has also been affected by consecutive canine distemper virus outbreaks (13). In this area, although not always consistently among different local guidelines, fox demographic trends are monitored annually, through the analysis of relative abundance indices such as passive surveillance results, den counts, hunting bags size and, more recently, spotlight counts performed simultaneously with red deer (14). The latter method has shown a potential for tracing large numeric fluctuations in fox populations, such as those that likely follow major epidemic outbreaks; however, uniform sets of data are often difficult to collect on a large scale and adjustments are required to develop more precise demographic predictors.

Echinococcus multilocularis in Europe is widely distributed in the temperate and cold areas of Northern and Eastern Europe; however, reports of the parasite are increasing in southern regions, as the Balkans, Greece and Italy (4, 15, 16). Among Member States of Europe, only Finland, Ireland, Malta and the United Kingdom have evidence of the continuous absence of *E. multilocularis* in their wild and domestic populations of definitive hosts (17). The EU recognises that *E. multilocularis* is of relevance to some Member States and that there is a need for surveillance within the Union to prevent it from spreading, including measures for movements of dogs, cats and ferrets, such as evidence of anti-cestodes treatment (18).

In Italy, *E. multilocularis* has been reported for the first time in red foxes shot and collected from 1997 to 2001 in the northern Italian Alps, close to the Austrian border (19), and it was later confirmed in foxes collected from 2001 to 2005 in the same area (20, 21). Other studies failed to find *E. multilocularis* in the

Central part of Italy (22, 23), while recently the parasite has been detected in shepherd dogs and wolves in the south-western Italian Alps, near the French border (24).

In 2011, several years after the first report of *E. multilocularis* in the northern Alps, the Italian Ministry of Health funded a novel monitoring of cestodes in red foxes (*Vulpes vulpes*), starting from the old focus, and extending the sampling area.

The aim of the present study is to report the results of the last seven years monitoring, describing the changes occurred over time and among different areas in the cestode community, with a particular focus on *E. multilocularis* and on the area in which this parasite appears historically present in North-eastern Italy.

Methods

Study area

The study area is located in north – eastern Italy (Wgs84 - Lon 10.396302 Lat 45.587717; Lon 13.983849 Lat 47.106623), bordering Austria and Slovenia and covering the territory of seven provinces (Bolzano, Trento, Belluno, Pordenone, Udine, Gorizia and Trieste) for about 2514 km^2 of mainly alpine land. Average altitude is approximately 1,230 m a.s.l., ranging from sea level in the south – eastern part (river Po Plain) to the highest values (up to 3,905 m a.s.l.) of central and western Dolomitic complexes. Overall monthly mean temperature (recorded from 1970 to 2000) ranges from − 2.16 °C in December to 15.69 °C in June (25).

To present our results in explicitly ecological terms, we refer to the framework given in Blasi and others (26), and therefore discretized our study area into six distinct Ecoregions (Fig. 1). Spatial discretization is based on potential natural vegetation (PNV), which is an expression of specific underlying climatic, biogeographic, physiographic and hydrographic factors and is considered to be relatively stable in time, providing a reference model on which to monitor the effects of natural dynamics. Ecoregions are briefly described in Table 1 (see Blasi and others (26), for further details).

Table 1
Ecoregions characterization. Precipitation and Temperature refer to mean annual range between stations. Ecoregion 1D (Italian part of Illyrian Province) was omitted since no reliable information is available.

Code	**Name**	**Elevation range (m above sea level)**	**Precipitation (mm)**	**Temperature (°C)**	**Bioclimatic Character**	**Land cover**
1A2a	**Pre-Alps**	45-2609	805–2628	2–14	Temperate semicontinental with subcontinental sectors near the Po Plain	Mainly natural-seminatural areas
1A2b	**Dolomiti and Carnia**	250–3263	690–2196	4–11	Temperate semicontinental with subcontinental sectors near the pre-Alps	Dominance of natural-seminatural areas
1A2c	**North-Eastern Alps**	200–4049	567–1302	5–12	Temperate semicontinental with widespread oceanic sectors in Alpi Lombarde	Dominance of natural-seminatural areas
1B1a	**Lagoon**	0–15	581–733	13–14	Temperate subcontinental	Dominance of agricoltural areas
1B1b	Central Plain	0-603	701–1346	11–14	Temperate semicontinental with widespread semicontinental-subcontinental sectors	Dominance of agricoltural areas

Sample collection

In the period 2012–2018, we collected faecal samples from red foxes legally hunted or found dead in the regions listed above, that were conferred to our laboratories for different purposes (as general diagnostics, rabies surveillance, oral vaccination monitoring, surveillance on *Trichinella* spp. parasites). For each fox, age (juvenile: foxes < 1 year old; adult: foxes > 1 year old), gender, date and geographical location of the finding/culling, were annotated. Each fox was then georeferenced according to the procedure outlined in Obber and others (14) (Fig. 1).

In accordance with Boitani and others (27), each fox was assigned, on the basis of the time of finding, to one out of six temporal classes of the biological cycle of the species:

1. January-February: mating period;

2. March-April: cubs birth period and early denning period, when cubs remain in the dens or in their immediate surroundings;
3. May-June: cubs spend time and become visible also outside the dens, seeking for food;
4. July-August: cubs begin to be more independent;
5. September-October: cubs begin to move out of their family group;
6. November-December: dispersal period, when cubs move to find, establish and mark a new territory.

Parasitological and molecular analysis

Faecal samples were kept frozen at − 80 °C for at least 72 h to inactivate eventual *Echinococcus* eggs and then tested using a filtration/sieving technique modified to enhance the likelihood to detect taeniid eggs (28). Briefly, 2 g of faeces were suspended in tap water, centrifuged and the sediment re-suspended in a zinc chloride solution and centrifuged. The supernatant was then filtered through sieves of different mesh sizes (41 µ and 21 µ) to concentrate taeniid eggs and exclude other parasite eggs. The presence of cestode eggs was verified using a bifocal, inverted microscope (Leica, Wetzlar, Germany).

Cestoda eggs were collected and DNA extracted by using DNeasy Blood & Tissue kit (Qiagen), according to the manufacturer’s instructions.

A multiplex PCR assay was performed using 3 couples of primers amplifying ND1 gene for *Echinococcus multilocularis* (394 bp) and 12S rRNA for both *E. granulosus* (117 bp) and *Taenia* spp. (271 bp) (29). Then, all the positive samples were amplified using a PCR assay targeting a fragment of cytochrome oxidase gene (460 bp) (30) and sequenced to confirm or determine the identity of the species. The PCR products were directly sequenced in a 16-capillary ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), using the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Sequence data were assembled and edited with SeqScape software v2.5 (Applied Biosystems, Foster City, CA, USA) and compared with representative sequences available in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi), using the Basic Local Alignment Search Tool (BLAST) (31) to identify the cestode species. For all PCR runs, DNA of pathogen-positive and negative samples (sterile water) served as control.

Statistical Analysis

Mapping and data spatial manipulation were carried out using ESRI ArcMap 10.5.1. (32). To highlight the differences over time and by ecoregion in cestode prevalence, laboratory outcomes (positives/negatives) were permuted by year and then analyzed using Fisher’s exact test (33) (Table 3 and Table 4). For the most represented cestode species (*E. multilocularis, T. crassiceps* and *T. polyacantha*), Pearson’s chi-squared test for linear correlation (34) was used to assess independence of outcomes from sample size and distribution. The Cochran-Armitage chi-squared test for trend in proportions (35, 36) was performed to evaluate the presence and intensity of a linear tendency in prevalence throughout the years. To test the importance of linear trend in the yearly prevalence variations, Pearsons’ and linear chi-squared were then compared and residuals chi-squared analyzed. Considering the importance of a consistent monitoring of

E. multilocularis, the seasonal prevalence and its possible trend along the study period have been analyzed, both for the whole study area and within the ecoregions where this zoonotic parasite was detected.

Table 3
Red foxes examined by years, overall cestode prevalence.

Year	Foxes tested	Positive to cestodes by coproscopy	Prevalence %
2012	1.063	67	6.3^{AB}
2013	474	18	3.8^{CDEF}
2014	248	19	7.7
2015	87	14	16.1^{AC}
2016	227	30	13.2^{BD}
2017	349	33	9.5^{E}
2018	424	36	8.5^{F}
Total	**2.872**	**217**	**7.5**
Differences of prevalence statistically significant (p < 0.01) are marked with equal letters.			

Table 4
Cestode positives and prevalence (%) by ecoregion. Statistically significant differences (p < 0.01) are marked with equal letters.

	Foxes examined	Cestode positives	Prevalence (%)
1A2a	680	32	4.7^{A}
1A2b	712	74	10.4^{AB}
1A2c	798	87	10.9^{AC}
1B1a	26	0	0
1B1b	609	22	3.6^{BC}
1D1a	47	2	4.2
Total	**2.872**	**217**	**7.5**

All statistical analysis was carried out using ‘stats’ package in R version 3.6.3 (37).

Results

Overall, 2.872 faecal samples of foxes were tested from 7 provinces (Fig. 1 and Table 2). Out of those, 217 (7.5%) tested positive for cestode eggs at coproscopy. According to the sampling year, the prevalence of cestodes ranged from a minimum of 3.8% in 2013 to a maximum of 16.1% in 2015 (Table 3).

Table 2
Foxes examined according to province in the northeast of Italy

Province	Foxes examined
Belluno	282
Bolzano	1.075
Trento	454
Gorizia	158
Trieste	33
Pordenone	79
Udine	791
Total	**2.872**

Considering the ecoregions, prevalence of cestodes ranged from a minimum of 3.6% in 1B1b to a maximum of 10.9% in 1A2c (Table 4).

Figure 2 shows total prevalence in foxes collected according to the bimestrial temporal classes.

Samples were distributed consistently throughout the year, although peaking late summer and autumn, in correspondence with the main hunting/control periods.

According to age and gender, the sample was structured as follows: 2,032 adults (71%), 533 juveniles (18%) and 307 undetermined (11%); 1,169 females (41%), 1,417 males (49%) and 286 undetermined (10%). The prevalence of cestodes as a whole was significantly higher in juveniles (12.57%) than in adults (6.25%) ($\chi^2 = 22.8$; $p < 0.01$), while was almost equal among females (8.1%), males (6.8%) and undetermined (8.7%).

PCR and sequencing successfully identified at a species level 178 (82.03%) out of the 217 positive samples. Eight species of cestodes were identified, with *T. crassiceps* (2.65%), *T. polyacantha* (1.98%), and *E. multilocularis* (1.04%) as the species most represented. The other species, *Mesocestoides litteratus, T. krabbei, T. serialis, T. taeniaeformis* and *D. caninum*, accounted for less than 0.2% each. In two cases, cestodes were identified at the genus level only, *Mesocestoides spp.* (0.38%) and *Taenia spp.* (0.10%) (Table 5).

Table 5
Cestode species identified by DNA sequence and prevalence (%) calculated on foxes positive for cestodes (coproscopy) and on the whole fox sample

Cestode species	**Positive foxes**	**%** **(n = 217)**	**%** **(n = 2.872)**
Taenia crassiceps	76	35.0	2.65
Taenia polyacantha	57	26.2	1.98
Echinococcus multilocularis	30	13.8	1.04
Taenia krabbei	2	0.92	0.07
Taenia taeniaeformis	2	0.92	0.07
Dipylidium caninum	2	0.92	0.07
Taenia serialis	1	0.46	0.03
Mesocestoides litteratus	5	2.30	0.17
Mesocestodes spp.	11	5.06	0.38
Taenia spp.	3	1.38	0.10

Representative sequences of each cestode species were submitted to GenBank (Accession Numbers will be provided as soon as they'll be available).

Co-infection was found in 11 foxes, which all harboured *E. multilocularis* plus *T. crassiceps* (8), *T. polyacantha* (1), *T. krabbei* (1) and *Taenia* spp. (1).

Considering ecoregions (Table 6), *E. multilocularis* was found only in two out of six (1A2b and 1A2c), while the other cestode species were found also in other ones. The spatial distribution of negative and positive foxes for *E. multilocularis* and cestodes is shown in Fig. 1.

Table 6
Cestode species identified by DNA sequence and relative prevalence (%) by ecoregion

Cestode species	**Ecoregion**					
	1A2a n = 680	1A2b n = 712	1A2c n = 798	1B1a n = 26	1B1b n = 609	1D1a n = 47
Echinococcus multilocularis	0	8 (1.12)	22 (2.75)	0	0	0
Taenia crassiceps	3 (0.44)	31 (4.35)	32 (4.01)	0	10 (1.64)	0
Taenia polyacantha	11 (1.62)	26 (3.65)	19 (2.38)	0	1 (0.16)	0
Taenia krabbei	0	0	2 (0.25)	0	0	0
Taenia serialis	0	0	0	0	0	1 (2.12)
Taenia taeniaeformis	0	2 (0.28)	0	0	0	0
Taenia spp.	1 (0.15)	0	2 (0.25)	0	0	0
Mesocestoides litteratus	3 (0.44)	0	1 (0.12)	0	1 (0.16)	0
Mesoceistodes spp.	2 (0.29)	3 (0.42)	6 (0.75)	0	0	0
Dipylidium caninum	1 (0.15)	0	1 (0.12)	0	0	0
Total	21	70	85	0	12	1

The shape of the distribution of main cestode species by bimester is similar to the one observed for cestodes collectively for both the whole study area (Fig. 3) and the focus ecoregions 1A2b and 1A2c (Fig. 4), showing higher values in the second half of the year.

Prevalence variations across the years of the study were evident and statistically significant on the whole study area (Fig. 5), both for cestodes collectively (Pearson $\chi^2 = 44.0$; $p < 0.01$) and for the three most consistent parasite species (*E. multilocularis*: Pearson $\chi^2 = 32.1$; $p < 0.01$; *T. crassiceps*: (Pearson $\chi^2 = 21.4$; $p < 0.01$ and *T. polyacantha*: (Pearson $\chi^2 = 21.2$; $p < 0.01$). Similar results were obtained when analyzing data from the two *E. multilocularis*-affected ecoregions, with the only exception of *T. polyacantha* (All cestodes: Pearson $\chi^2 = 16.5$; $p < 0.05$; *E. multilocularis*: Pearson $\chi^2 = 20.1$; $p < 0.01$; *T. crassiceps*: Pearson $\chi^2 = 14.1$; $p < 0.05$ and *T. polyacantha*: Pearson $\chi^2 = 8.5.2$; $p > 0.05$) (Fig. 6).

A statistically significant linear trend pattern in prevalence across the years was also evidenced on the whole study area for cestodes collectively (Armitage $\chi^2 = 25.6$; $p < 0.01$), for *E. multilocularis* (Armitage $\chi^2 = 27.1$; $p < 0.01$) and *T. polyacantha* (Armitage $\chi^2 = 8.1$; $p < 0.01$). When analyzing data in the two *E. multilocularis* affected ecoregions, a linear trend was still statistically detectable for cestodes collectively (Armitage $\chi^2 = 7.0$; $p < 0.01$) and *E. multilocularis* (Armitage $\chi^2 = 19.3$; $p < 0.019$). However, it must be pointed out that, both on the whole and in the focus areas, residual χ^2 exceeded the respective critical tabulated value of χ^2 except for *E. multilocularis*, for which the residual value was lower than the tabulated one. Therefore, only for this parasite a linear trend could reasonably account for most of the inter-annual variation in prevalence.

Discussion

Our results confirmed the presence of *E. multilocularis* in red foxes of northeast Italy and highlighted a tendency of increase in prevalence through the years. In previous studies (19–21), *E. multilocularis* positive foxes occurred close to the Austrian border, leading to the hypothesis of a transboundary focus of echinococcosis in the Alps. However further research using a multi-locus microsatellite analysis, supported the hypothesis of an autochthonous focus in Italy rather than a recent incursion of infected foxes from abroad (38, 39). Within a fox population, the majority of animals migrating in new territories are usually juveniles, which disperse from their native territory and spread the parasite marking the new home range with faeces (1). The higher prevalence of *E. multilocularis* in juveniles compared to adults, found also by other Authors (40), is likely to be the reason for the seasonal distribution pattern observed in cestode prevalence: actually, in the early months of the year juveniles are absent. At the same time, this helps to identify juveniles as the main drivers of a possible expansion of the focus, since adult foxes might in fact acquire partial immunity after repeated exposure (40). The red fox is commonly viewed as a “pest” species (41); as game value is considered low, wildlife managers often resort to additional control measures such as limiting trophic resources and/or culling during the breeding season to curtail demographic expansion. Numeric control measures are also undertaken in order to address predation rate upon endangered (white partridge, capercaillie, mountain hare), game (grey partridge, common pheasant, brown hare, quails) and domestic (rabbits, poultry) species. However, different studied have shown how control measures may turn out to be ineffective or even counterproductive in both target species demography and epidemiological terms, especially when applied to insufficient knowledge of ecological priors (e.g. 42). Further investigations should address particularly the role of dispersal pathways on fox population response to sudden demographic changes, and the potential epidemiological risk associated with it, to inform future management actions.

Another interesting issue is the significant increasing trend in prevalence showed by *E. multilocularis* across the last years, both in general and specifically in the affected ecoregions. Two important epidemics occurred in the fox population in northeast of Italy, namely subsequent epidemics of canine distemper virus (since 2006) and a severe rabies epizootic from 2008 to 2011 (12, 13). These events have likely affected the local red fox populations in terms of both density and age structure, and might have affected as well the prevalence of *E. multilocularis* and its apparent upsurge in 2015. Other ecological changes, such as temporal variations of the intermediate host density, could also explain the significant variability of the echinococcosis positivity through years. Finally, the increasing trend evidenced may be partially due to a likewise increasing attention paid to this zoonotic parasite over the years: however, although there is no doubt about this increasing interest, in the years of our study the methods have been kept consistent.

Compared to previous studies, the mean prevalence of *E. multilocularis* in the Bolzano territory (2.8%) is substantially lower. Indeed, in red foxes collected from 2001 to 2005 and tested through PCR, *E. multilocularis* was found in 12.9% of foxes from Bolzano and 2.9% of foxes from Trento province (21). On the contrary, in our study, we did not find positive foxes in Trento province and the prevalence of *E. multilocularis* even in the most affected ecoregion (1A2c) was 2.75%. Prevalence determined by PCR on cestodes eggs isolated from the faeces (egg-PCR) is likely to be underestimated: Otero-Abad and others (43) calculated for the same egg-PCR technique we used, a good specificity of 93.4% and a moderate sensitivity of 54.8%. Our prevalence of *E. multilocularis* is very similar to the 2.6% found in the bordering Slovenia in 2010 (15), but it is much lower than that recorded in endemic areas of Europe. There, *E. multilocularis* may reach in red foxes very high prevalence locally, such as 28.5% in Denmark (44), 25.6% in Poland (45), and in general a prevalence over 10% in eastern countries (reviewed in Oksanen (3)), where human cases are annually reported (46). Moreover, in such areas, due to the high prevalence of the parasite, the positive predictive value of diagnostic tests will probably be much higher than in our territory, in which *E. multilocularis* is instead a patchy and rare pathogen with very low prevalence. Therefore, results in areas as wide as north eastern Italy should be considered with particular caution, all the more reason considering that on such a scale a wide variability among the yearly sample sizes seems unavoidable. In our context, higher prevalence values can be found at a very local level (e.g. the Alta Val d'Isarco district, an area covering about 650 km^2, where prevalence reaches 11%), and in the absence of specific and local models to predict the potential reach of the parasite, we suggest results to be presented on a scale determined by ecological constrains.

The incidence of human cases of *E. multilocularis* in Europe is approximately proportional to the prevalence in the definitive hosts, and showed a significant increasing trend in the years 2008–2016 (46). In endemic areas, the increase of fox density close to villages may put at risk of infection dogs and consequently increase the risk for humans. Indeed, in an endemic area of France, *E. multilocularis* was detected by PCR in 35%, 11% and 7% of fox, dog and cat faeces, respectively, collected in kitchen gardens (47). Italy has never officially reported a confirmed autochthonous case in humans, but at the end of the nineteenth century, human cases were recorded in Pusteria valley and from Bressanone, in Bolzano province (48, cited in 21). Besides *E. multilocularis*, the other species of cestodes identified in our study constitute part of the typical parasite fauna of red foxes in Italy (22, 49–56). The differences of prevalence with respect to other Italian and European regions are likely to be modulated by the distribution and abundance of the local intermediate hosts (57, 58). *Taenia krabbei* represents an exception, since it is reported in the artic fox (57, 59) but rarely in the red fox (60) and it is more common in wolves, Italy included (24, 61, 62). Very recently, cisticercosis due to *T. krabbei* has been identified for the first time in Italy in two hunted roe deer (*Capreolus capreolus*) and a dog of the Italian northern Apennines (63). However, the taxonomic status of *T. krabbei* is still uncertain, as mitochondrial DNA analyses of morphologically similar isolates from northern Europe, revealed the presence of cryptic species within *T. krabbei* (64).

Among the species of cestodes found in our study, some have been appointed as zoonotic as well, i.e. *T. crassiceps. T. taeniaeformis* and *T. serialis*, causing cysticercosis, strobilocercosis and coenurosis, respectively (65). However, cases on humans are rare worldwide and do not represent a major public health concern.

Apart from *Dipylidium caninum* and *T. krabbei*, which recognise fleas and large ungulates as intermediate hosts, respectively, all the cestode species found in our study have rodents as intermediate hosts, evidently still an important component of the diet of the red fox in this ecological context. Intensity of predation on small rodents could be as well one of the main factors allowing the persistence of *E. multilocularis*, although it could only partially account for its still limited range. At the same time, ecoregions appear as a tool too gross to deepen the ecology of the host-parasite-environment system.

Conclusions

Our study confirms the presence of a low prevalence focus of *E. multilocularis* in red foxes of northeastern Italy, still confined in the area near the Austrian border. Given the persistence of the focus through the years, we recommend at a local level an informative campaign on how to behave to avoid contamination, targeted at people living in the area, especially hunters, dog owners, forestry workers and other categories at risk.

In Italy, *E. multilocularis* in foxes as well as alveolar echinococcosis in intermediate hosts, including humans, should be approached as rare diseases. Consequently, rather than screening at a great scale, further work should focus on the ecology of this disease in the definitive and intermediate hosts and in the environment on a small scale, in the areas where its presence has been already demonstrated, with the final aim to understand which are the factors that allow the persistence of this parasite.

Declarations

Ethics approval and consent to participate

Not applicable. All samples originated from wild red foxes found dead or legally hunted.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

CVC and GC conceived the study; DD, KT, MB, RC and CVC organized the local collection of foxes; FO and PB built the original dataset and maps; GC, PD, SR, GDR, SG and SO performed coprological, biomolecular and sequence analyses. All authors MI, SV, DR, AA, AC, DA contributed to interpretation and critical review. All authors read and approved the final manuscript.

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Figures

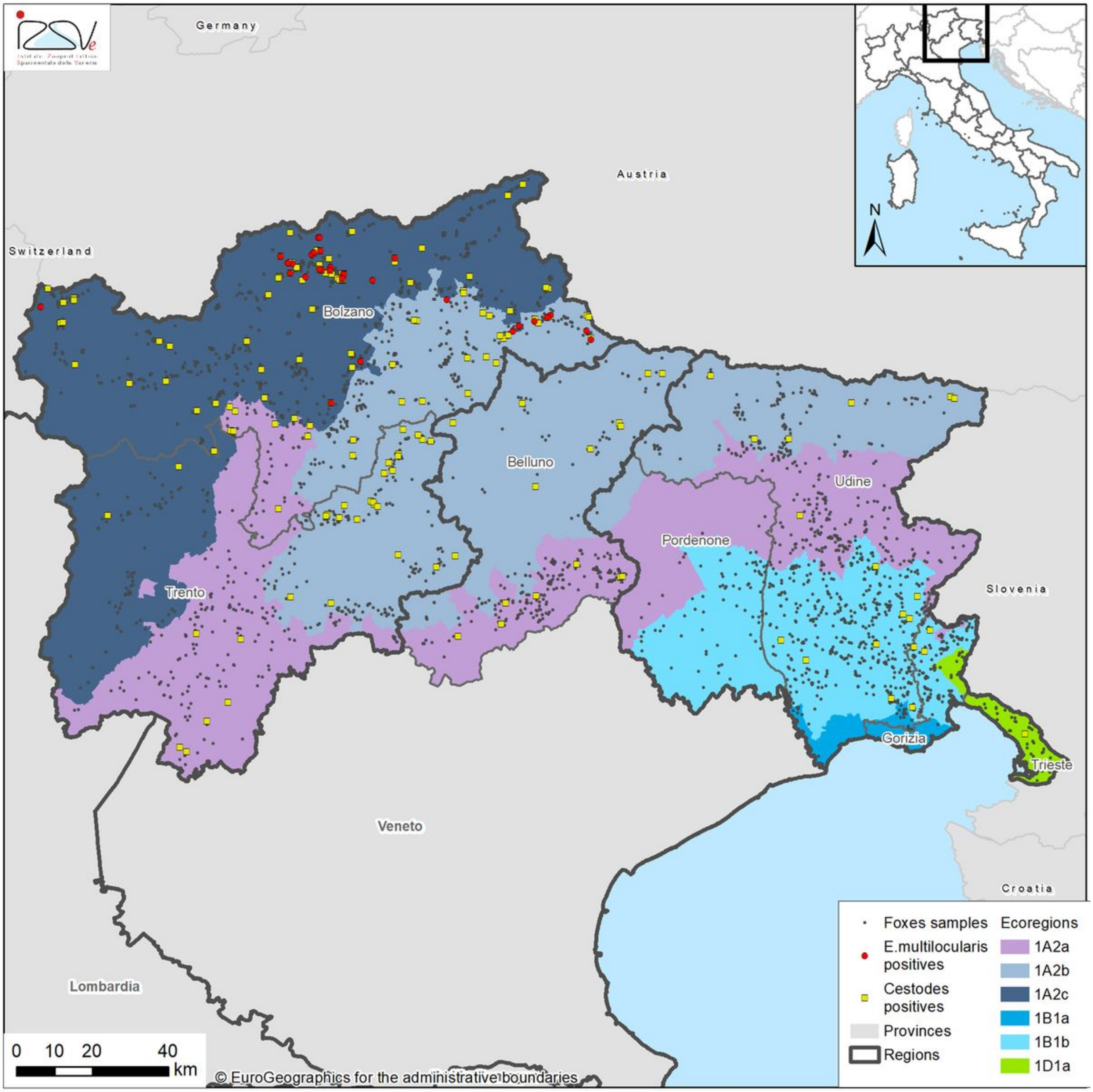

Figure 1

Distribution of cestode positives and E. multilocularis positive foxes (whole sample) according to province and ecoregions in the northeast of Italy.

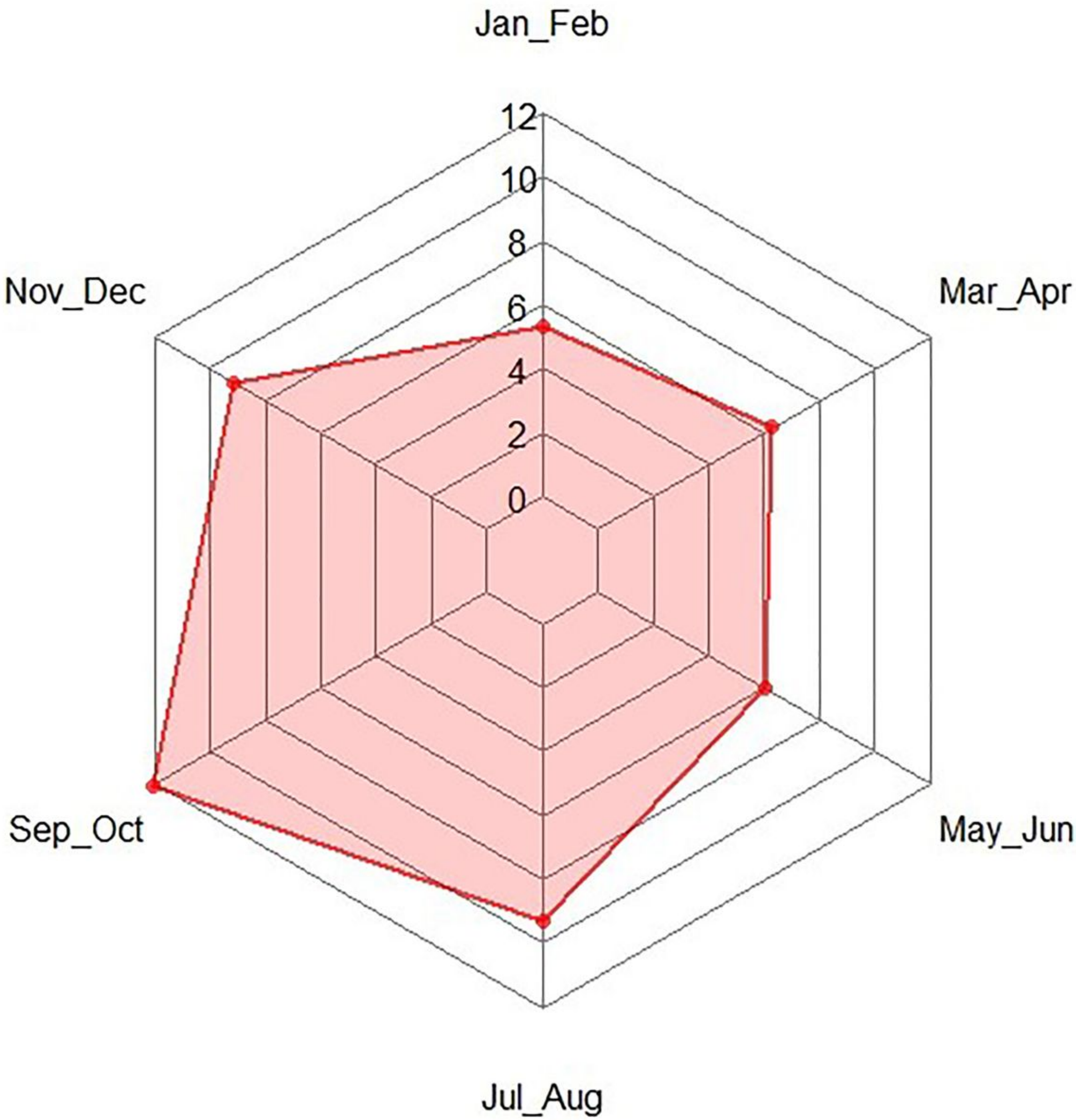

Figure 2

Overall cestode prevalence (%) by bimester.

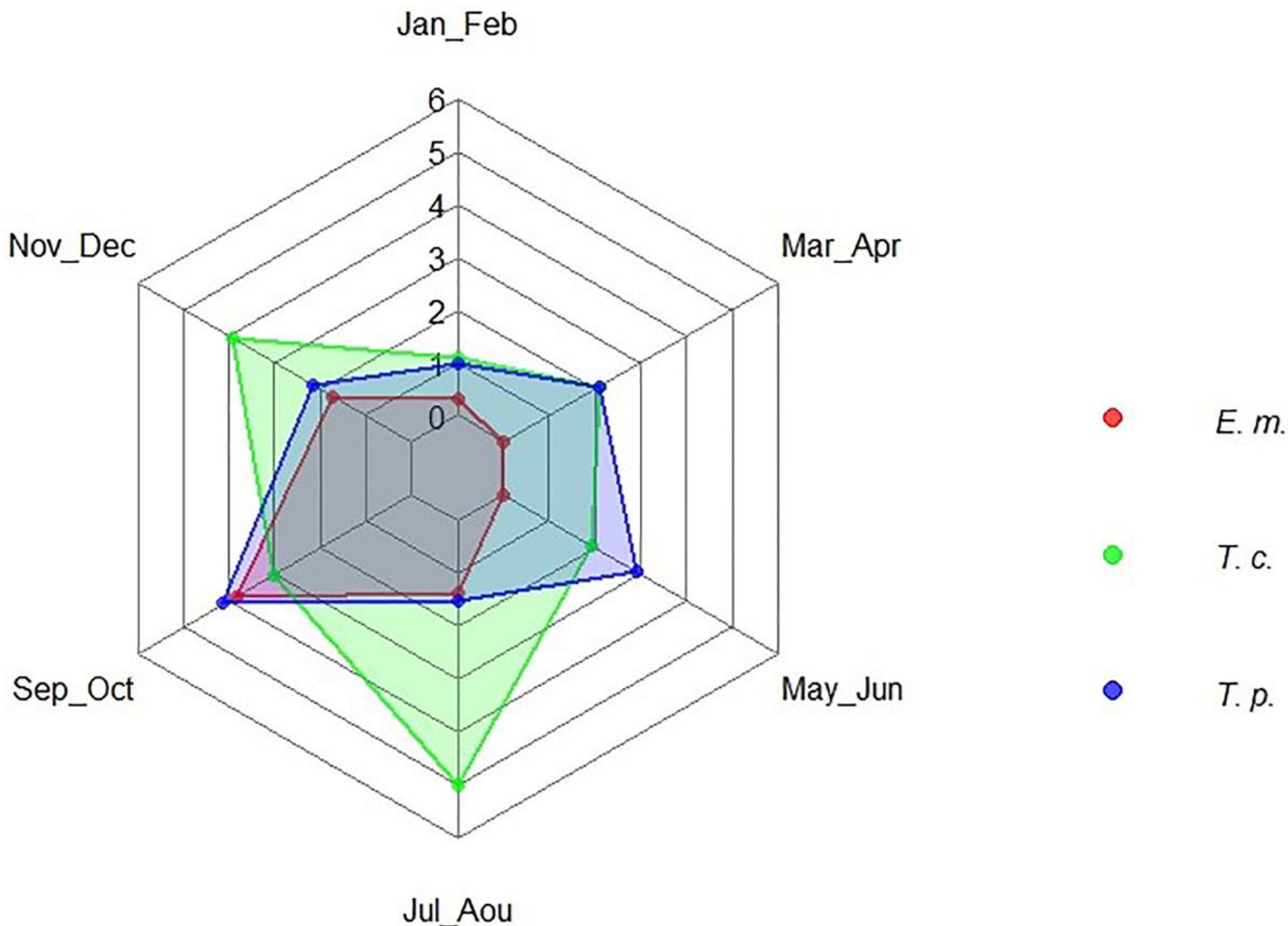

Figure 3

E. multilocularis (E. m.), T. crassiceps (T. c.) and T. polyacantha (T. p.) overall prevalence (%) by bimester.

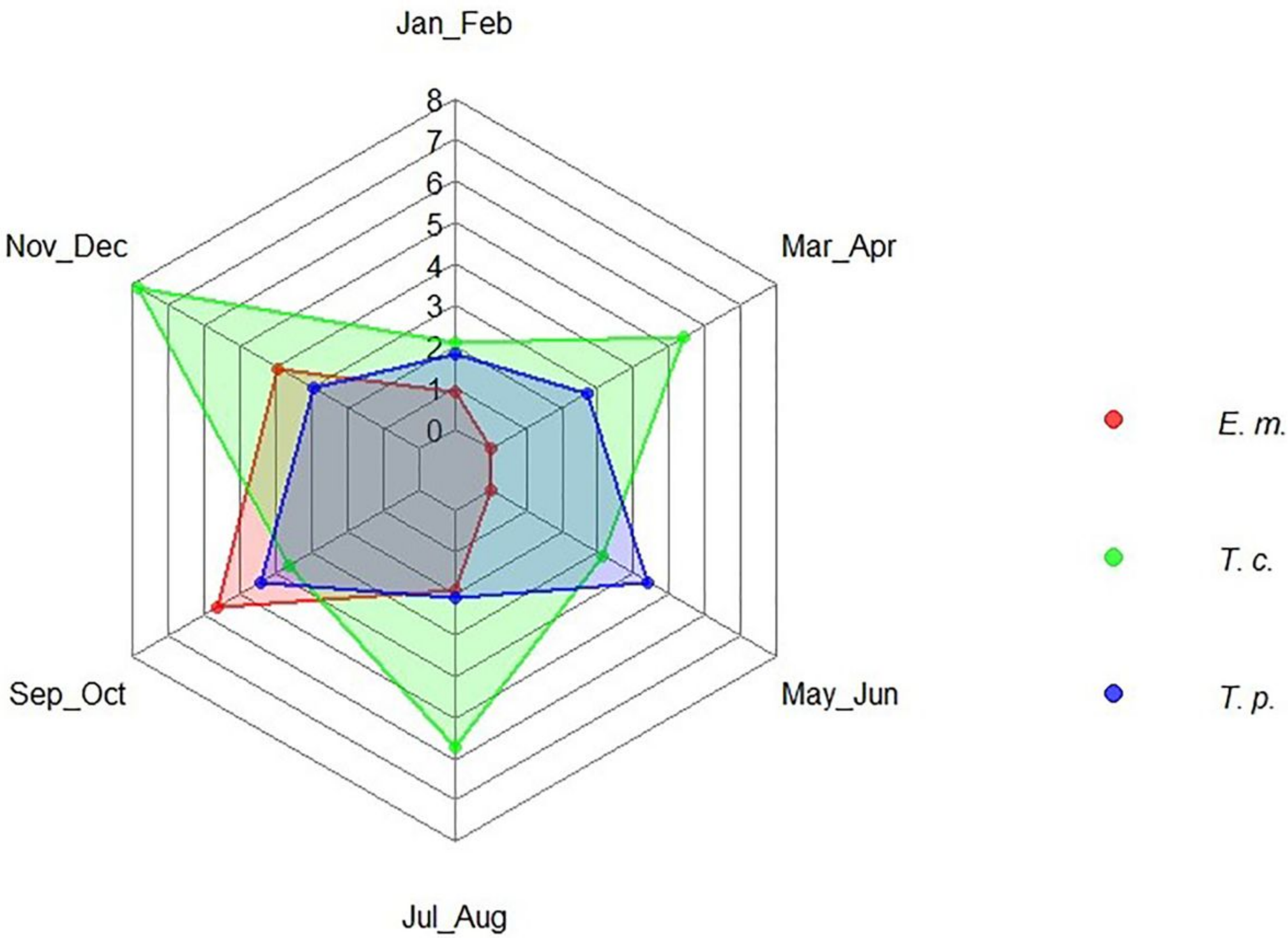

Figure 4

Joined E. multilocularis (E. m.), T. crassiceps (T. c.) and T. polyacantha (T. p.) prevalence (%) in ecoregions 1A2b and 1A2c by bimester.

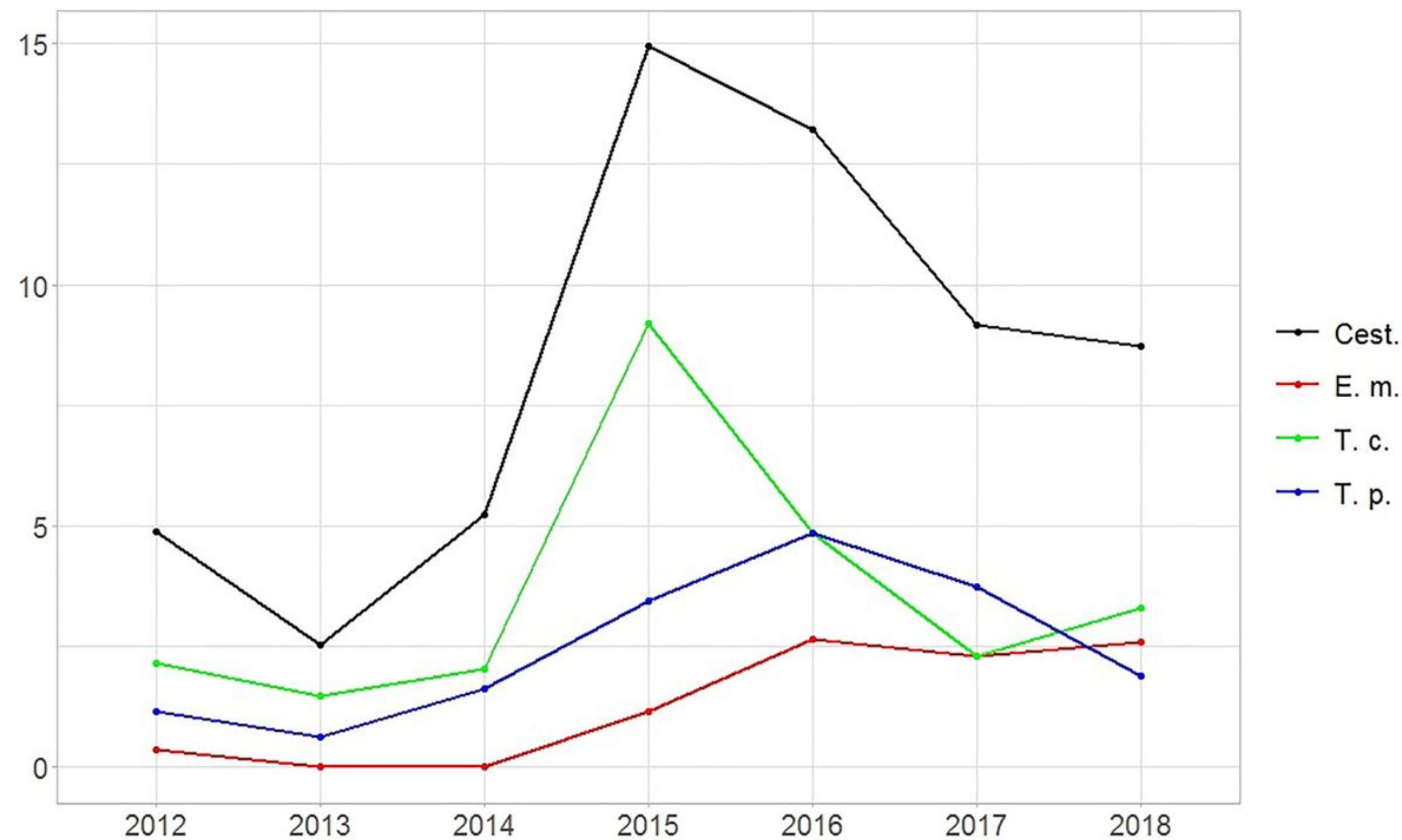

Figure 5

Cestodes (Cest.), E. multilocularis (E. m.), T. crassiceps (T. c.) and T. polyacantha (T. p.) overall prevalence (%) in the study area by year.

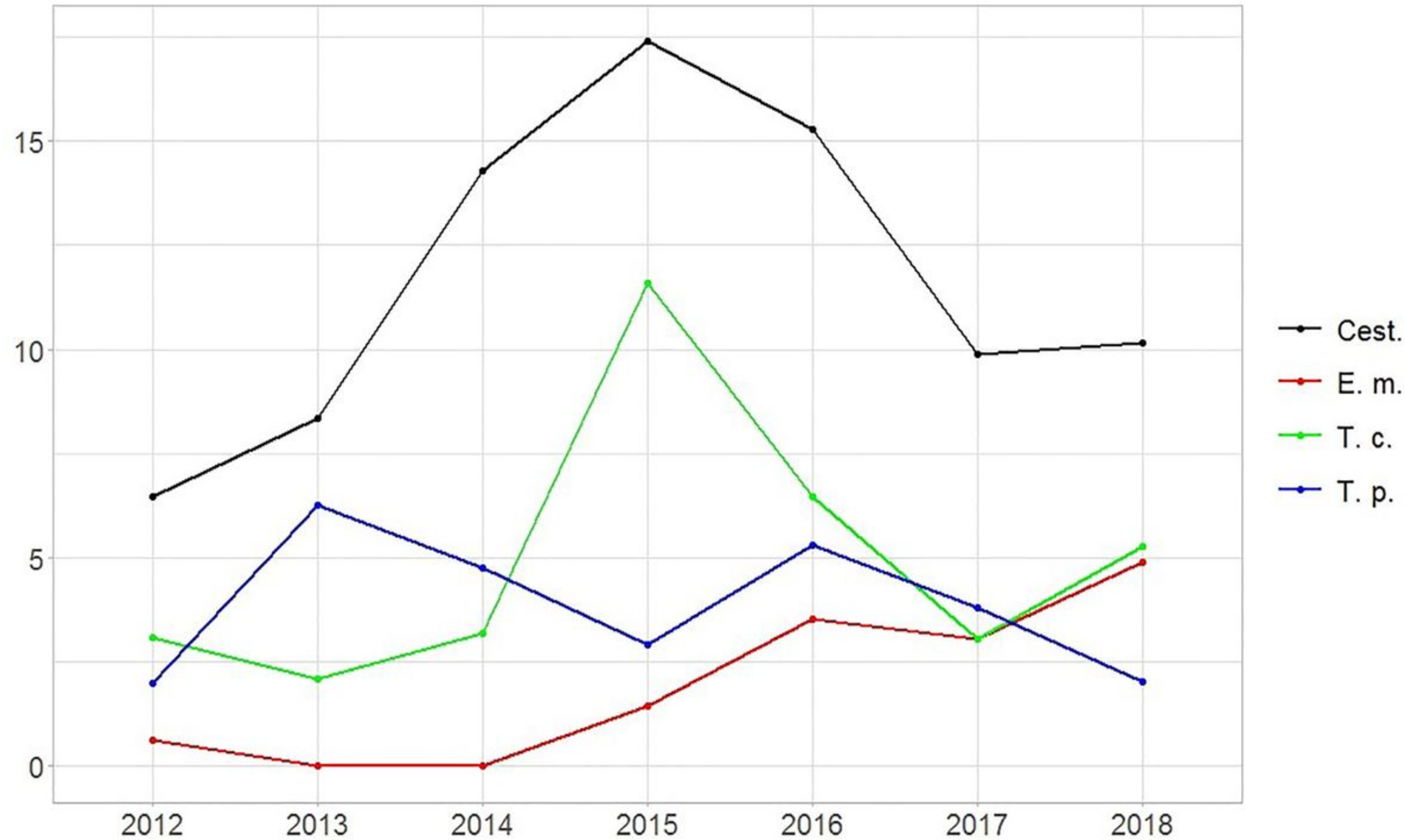

Figure 6

Cestodes (Cest.), E. multilocularis (E. m.), T. crassiceps (T. c.) and T. polyacantha (T. p.) overall prevalence (%) in ecoregion 1A2b and 1A2c by year.