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Echinococcus multilocularis in red foxes (*Vulpes vulpes*) of the Italian Alpine region: is there a focus of autochthonous transmission?

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Abstract

Alveolar echinococcosis, caused by the metacestode of *Echinococcus multilocularis*, is a zoonosis with a wider distribution area than described in the past. Fox populations living in the Alpine regions of Italy had been considered free from this parasite until 2002, when two infected foxes (*Vulpes vulpes*) were detected in the Bolzano province (Trentino Alto Adige region) near the Austrian border. The aim of this work was to evaluate the prevalence of infection in red fox populations from five Italian regions. A modified nested PCR analysis was used to detect *E. multilocularis* DNA in faecal samples. Amplicons were confirmed by sequencing. Of 500 faecal samples from foxes shot in Valle d'Aosta ($n=57$), Liguria ($n=44$), Lombardy ($n=102$), Veneto ($n=56$), and Trentino Alto Adige ($n=241$) regions, 24 animals, all from the Trentino Alto Adige region, were found positive. Twenty-two positive animals originated from the Bolzano province and two positive animals from the Trento province. Several localities of the Bolzano province, in which positive foxes were detected, are the same as those where alveolar echinococcosis had been described in humans in the second half of the 19th century, suggesting an old endemicity for the investigated area, which is adjacent to endemic areas of Austria. Therefore, the question arises if we are observing an increase and expansion of foci, or if the new records are due to the more sensitive and specific methods used to detect the worm DNA.

Keywords: Alveolar echinococcosis; *Echinococcus multilocularis*; Red fox; Italy; Alps; Prevalence; PCR

1. Introduction

Human alveolar echinococcosis is caused by the larval stage of the tapeworm *Echinococcus multilocularis* and is considered one of the most pathogenic helminthic zoonoses (Vuitton et al., 2003). In Europe, the natural cycle of the parasite is predominantly sylvatic, involving rodents of several genera (e.g. *Arvicola*, *Mus*, *Microtus* and *Clethrionomys*) as intermediate hosts and the red fox (*Vulpes vulpes*) as the principal definitive host (Eckert and Deplazes, 2004).

Until the end of the 1980s, the endemic areas of *E. multilocularis* were reported to be confined to parts of Austria, Switzerland, Germany, and France (Eckert et al., 2000; Vuitton et al., 2003). During the 1990s and at the beginning of the 2000s, the prevalence of infection in foxes increased in several endemic areas, and, at the same time, new foci were detected in Belgium (Brochier et al., 1992), Poland (Malczewski et al., 1995), the Czech Republic (Kolarova et al., 1996), Luxembourg (Losson et al., 2003), the Netherlands (van der Giessen et al., 1999), the Slovak Republic (Dubinsky et al., 1999) and Hungary (Sreter et al., 2003). Investigations conducted on red foxes of the Alpine region of Italy were negative (Iori et al., 1990; Stancampiano et al., 1998) up to 2002, when *E. multilocularis* worms were detected for the first time in two red foxes from an

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Italian Alpine area very close to the border with Austria (Manfredi et al., 2002).

For diagnosis in definitive hosts the sedimentation and counting technique (SCT) has been considered the gold standard (Eckert et al., 2001), but the disadvantage of this technique is the need of logistical requirements for maintaining and processing fox carcasses (e.g. a room for necropsy, −80 °C dedicated freezers), a skilful examiner and a time consuming method. Serological screening is considered unsuitable for the diagnosis, because of a poor relationship between the presence of the antibodies and tapeworms (Deplazes and Eckert, 1996). Coproantigen ELISA and copro-PCR techniques have been developed to detect *E. multilocularis* infections (Mathis et al., 1996; Monnier et al., 1996; Dinkel et al., 1998; Sakai et al., 1998; Deplazes et al., 1999; van der Giessen et al., 1999). For the detection of coproantigens in animal populations with an expected low prevalence for this parasite, the ELISA has high negative and low positive predictive values for the infection (Deplazes et al., 2003).

Since the prevalence of *E. multilocularis* in definitive hosts is an important parameter to estimate the potential risk of infection for humans, this study was designed to assess the prevalence of infection in the red fox population from Alpine regions of Italy, by a modified nested PCR analysis.

2. Materials and methods

2.1. Sample collection and DNA extraction

Between 2001 and 2004, red foxes killed by hunters were collected in different areas from 200 to 2000 m above sea level (average 1100 m above sea level) of the Alpine regions of Italy. Carcasses were individually labelled with an identification number, sex, locality of origin, altitude and date of killing. Foxes were skinned and sent by hunters to regional institutions, where the intestines were collected by each animal. A faecal sample was collected from the rectum and preserved in a 4 ml vial. Both the intestine and the vial were labelled with a code and frozen separately at −20 °C. Then the frozen intestines and faecal samples were sent to the University of Milan, where they were first frozen at −80 °C for 2 weeks to kill the embryos of *E. multilocularis* present in eggs for safety reasons, and then stored at −20 °C until examination. A total of 500 intestines and faecal paired samples were collected and analysed, originating from the provinces: Aosta (57) (Valle d'Aosta region); Imperia (20) and Savona (24) (Liguria region); Bergamo (102) (Lombardy region); Belluno (56) (Veneto region); Bolzano (171) and Trento (70) (Trentino Alto Adige region) (Fig. 1).

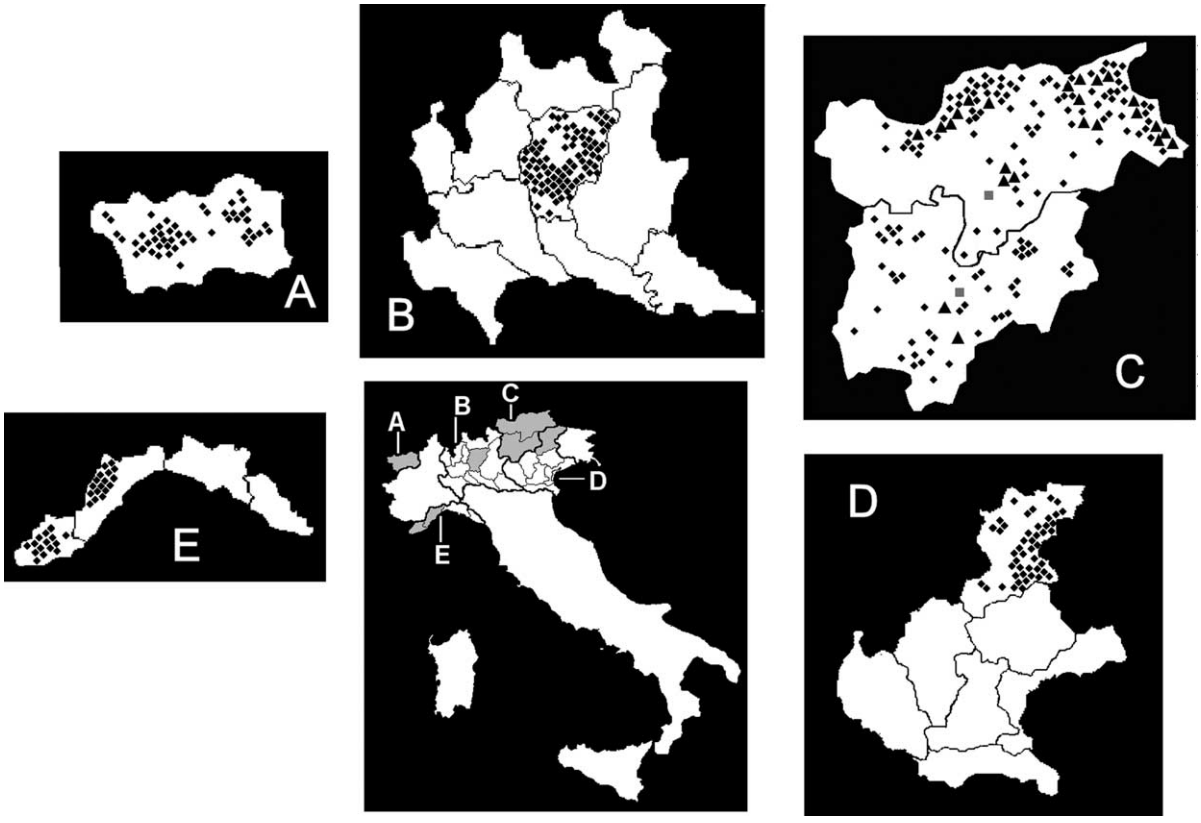

Fig. 1. Locality of origin of red foxes (*Vulpes vulpes*) examined for *Echinococcus multilocularis*. In grey, provinces of origin of examined animals. A, Valle d'Aosta region; B, Bergamo province, Lombardy region; C, Bolzano and Trento provinces, Trentino Alto Adige region; D, Belluno province, Veneto region; E, Imperia and Savona provinces, Liguria region. Thick lines represent regional borders, thin lines represent province borders. Black triangles represent positive foxes in Trentino Alto Adige region; black dots one or more negative foxes; grey dots the cities of Trento and Bolzano.