

Detection of a high-endemic focus of *Echinococcus multilocularis* in red foxes in southern Denmark, January 2013

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Citation style for this article:

Enemark HL, Al-Sabi MN, Knapp J, Staahl M, Chriél M. Detection of a high-endemic focus of *Echinococcus multilocularis* in red foxes in southern Denmark, January 2013. *Euro Surveill.* 2013;18(10):pii=20420. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20420>

Article submitted on 01 March 2013 / published on 07 March 2013

The Danish surveillance programme for *Echinococcus multilocularis* was initiated in September 2011, and so far 679 wild carnivores have been examined. In April 2012, one infected fox was detected in Højer near the Danish-German border, and in January 2013 three additional foxes from the same area were found infected. Local prevalence in the area was 31% (four of 13 foxes) which is a new epidemiological situation calling for re-evaluation of the national risk management.

As part of the surveillance for the fox tapeworm *Echinococcus multilocularis*, one red fox (*Vulpes vulpes*) was found positive in April 2012. The animal had been shot in November 2011 in the Højer area, Jutland in the southern part of Denmark, less than 10 km north of the border between Denmark and Germany [1]. In January 2013, *E. multilocularis* was detected in a further three foxes, one had been shot in September 2012, the other two in November 2012. A total of 13 foxes from this location have been examined corresponding to a local prevalence of 31% (95% confidence interval (CI): 7–55) in foxes. These were the first findings of *E. multilocularis* in mainland Denmark.

Background

E. multilocularis is endemic in large parts of Europe, and has been detected with increasing prevalence and geographical spread during the last decades, including in countries bordering Denmark, such as Germany [2], the Baltic States [3], and most recently Sweden [4]. Humans may be accidental intermediate hosts and develop alveolar echinococcosis, one of the most severe zoonotic infections in the northern hemisphere. Infections in humans are rare but cause considerable public health concern due to treatment costs and high mortality if left untreated [5]. In Denmark, infection with *E. multilocularis* is notifiable in all animal species, but not for human cases.

E. multilocularis was discovered for the first time in Denmark in 2000 in three of 1,040 foxes (0.3%). However, all infected foxes were from the Copenhagen

area (Zealand) which corresponds to a local prevalence of 0.9% (three of 340 foxes) [6]. No additional national surveillance has been conducted in wild carnivores, but one of 169 clinical samples from domestic Danish cats, submitted to a diagnostic German laboratory for routine analyses in 2004–05, was found positive [7].

Surveillance of *Echinococcus multilocularis* in wild carnivores, 2011–13

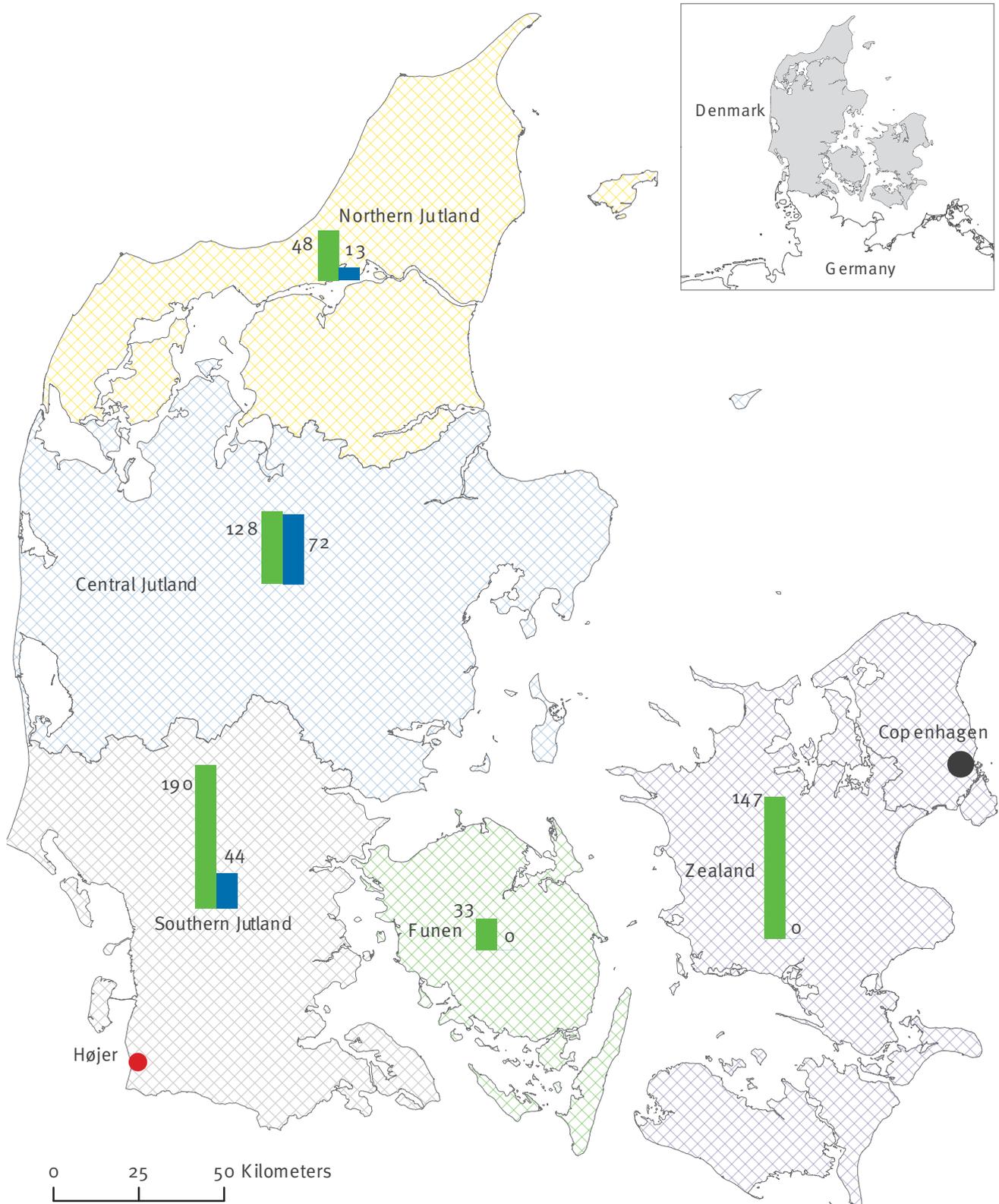
Following the detection of *E. multilocularis* in a Swedish fox in 2011 [4], a surveillance programme for *Echinococcus* in wild carnivores was initiated in Denmark in September 2011. Included in the surveillance were road-killed and hunted animals collected by the Danish Nature Agency or by voluntary hunters. To date, a total of 679 wild carnivores, namely 546 foxes, 129 racoon dogs (*Nyctereutes procyonoides*), three badgers (*Meles meles*) and one wolf (*Canis lupus*), have been examined by the sedimentation and counting technique [8]. The geographical origin of the tested foxes and racoon dogs is shown in Figure 1.

The first positive fox was shot in November 2011 in Jutland close to the village Højer, 8 km north of the border to Germany (Figure 1), and tested positive in April 2012. The fox was an adult male harbouring 20 adult tapeworms. In addition to morphological identification, these worms were analysed by PCR amplification and sequencing of the 12S rRNA gene [9], revealing a 200 bp product that was 100% identical to *E. multilocularis* sequences in GenBank, e.g. JX068642. Subgenotyping by fragment size analysis of the EmsB microsatellite marker [10] was done to compare the genetic profile with other European isolates (Figure 2). The results revealed no close relationship to other isolates analysed so far.

In January 2013, three additional foxes from the same area, shot within a radius of 10 km, were detected positive for *E. multilocularis* by the sedimentation and

FIGURE 1

Geographical distribution of all foxes and raccoon dogs analysed as part of the Danish *Echinococcus multilocularis* monitoring programme, September 2011–January 2013 (n=675)



Green bars: foxes. Blue bars: raccoon dogs.

The red circle in the bottom left corner of the map indicates the area where four infected foxes were shot from November 2011 to November 2012.

counting technique. These foxes, all adult females, were shot in September and November 2012, and harboured two, seven and 27 adult tapeworms, respectively. Molecular analysis of these worms is ongoing. Until now a total of 13 foxes (of which four were positive) and three racoon dogs (all negative) from this area have been examined for infection with *E. multilocularis* corresponding to a local prevalence of 31% (95% confidence interval (CI): 7–55) in foxes. Based on the preliminary surveillance data, the countrywide prevalence of *E. multilocularis* is 0.7%. So far infection with *E. multilocularis* has not been detected in wild carnivores other than foxes in Denmark.

Discussion

The Danish *E. multilocularis* isolate did not cluster closely with any other European isolates of fox origin sub-genotyped until now. Hence, introduction from neighbouring countries cannot be documented on the present basis.

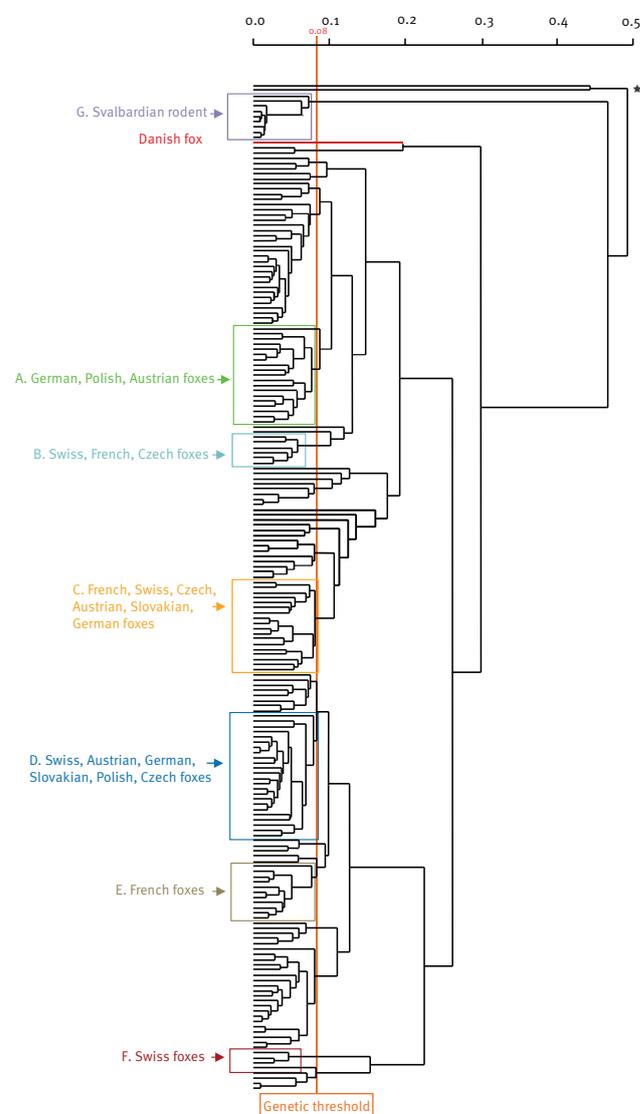
E. multilocularis was first detected in Denmark more than a decade ago [6], but has never before been detected outside Zealand. However, as no surveillance was in place, the parasite may not have been detected. The temperate climate of Denmark allows the survival of *E. multilocularis* eggs for extended periods, and rodents, implicated as intermediate hosts of the parasite in other European countries, are prevalent [11]. Thus, conditions for the establishment and spread of infection are present, although alveolar echinococcosis in intermediate and aberrant hosts has not yet been detected, and there is no information on any autochthonous human cases.

The samples collected for the present study were representative of the whole country. Nevertheless, the current prevalence of *E. multilocularis* in Danish wildlife is based on analyses of a relatively low number of samples and it is therefore too early to conclude whether there is a general increase in the prevalence on a national level. Even in low-endemic regions, local foci with high prevalence of *E. multilocularis* in foxes are known [12,13]. A consistently high prevalence of 35–65% of *E. multilocularis* has been registered in foxes in endemic European countries, where human cases appear, and foxes are believed to be responsible for most of the environmental contamination with *E. multilocularis* eggs [14]. Thus, a local prevalence of over 30% in foxes is worrying, and even if based on a small number of foxes, poses an increased risk of transmission to humans as well as dogs and cats. On this background the Danish Veterinary and Food Administration has recommended since 18 February 2013 that dogs in Tønder municipality (i.e. southern Denmark) that are allowed to roam freely in the countryside (including hunting dogs), are dewormed regularly with praziquantel every fourth week.

Due to the long incubation time of alveolar echinococcosis, an increased prevalence of *E. multilocularis* in the fox population will not immediately be reflected

FIGURE 2

Dendrogram of euclidean distance amongst *Echinococcus multilocularis* worms from foxes in Austria, the Czech Republic, Denmark, France, Germany, Poland, Slovakia and Switzerland, and rodents from Svalbard (Norway) (n=193 + two out-group samples)



Out-group samples (*Echinococcus granulosus*) are marked with a star. Samples with a genetic distance > 0.08 are considered as different. Groups A to D are examples of multi-origin clusters and groups E to G are single-origin clusters.

in an increased incidence of alveolar echinococcosis among humans [8]. However, as alveolar echinococcosis spreads in a tumour-like manner it can be misdiagnosed as liver cancer. Alveolar echinococcosis should therefore be considered as a differential diagnosis, and may become increasingly important in the future.

Worm burdens detected in Danish foxes so far have been low, which is a diagnostic challenge. On the other hand, fewer worms excrete fewer eggs and these foxes probably do not contaminate the environment as much as foxes with large worm burdens. Nevertheless, a local prevalence of this magnitude emphasised the need for re-evaluating risk management and risk communication in the region, and calls for increased awareness among veterinarians as well as physicians.

Acknowledgements

Danish hunters and the Danish Nature Agency are thanked for supplying carnivores for the surveillance study. Cynthia D Juel, Boi-Tien T Pham and Leif B Eiersted are acknowledged for skilled technical assistance, G Umhang for genotyping of the worms, and B Kristensen for making the map in figure 1. The study was financed by the Danish Veterinary and Food Administration.

Conflict of interest

None declared.

Authors' contributions

Heidi Enemark was responsible for design and conduction of the study and led the writing of the rapid communication. All authors provided contribution to the manuscript and approved the final version. Mohammad Nafi Al-Sabi performed the sedimentation and counting analyses, Jenny Knapp did the microsatellite sub-genotyping, Marie Staahl was in charge of the PCR analysis, and Mariann Chriel coordinated animal sampling.

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