

## Estimated prevalence of *Echinococcus multilocularis* in raccoon dogs *Nyctereutes procyonoides* in northern Brandenburg, Germany

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**Abstract** Human alveolar echinococcosis, caused by the larval stage of the small fox tapeworm, is a lethal zoonotic infection if left untreated. *E. multilocularis* is distributed in the Northern Hemisphere and lives in the small intestines of carnivores, mainly canids. The main definitive host of *E. multilocularis* in European countries is the red fox *Vulpes vulpes* and in the last ten years new endemic areas for the parasite in Central Europe have been identified. In some areas, for instance in Germany, the raccoon dog *Nyctereutes procyonoides* - a spreading neozoon - must be regarded as an additional definitive host for *E. multilocularis*. In 2001 this parasite was found for the first time in raccoon dogs in the Federal State of Brandenburg, Germany. Between 2000 and 2008, 1,252 raccoon dogs from Brandenburg were examined by the Intestinal Scraping Technique. The majority of samples were obtained in five northern counties and all 60 animals that tested positive for *E. multilocularis* were located there. The estimated true prevalence calculated by a beta-binomial- model ranged from 6%–12% [Current Zoology 57 (5): 655–661, 2011].

**Keywords** Zoonosis, Invasive species, Echinococcosis, Germany, Raccoon dog

The 2–4 mm sized adult small fox tapeworm *E. multilocularis* lives in the small intestines of carnivores, mainly canids, which are the definitive hosts of *E. multilocularis* and shed the eggs (oncospheres) of the parasite in their faeces. Several rodent species, in particular voles, the natural prey of carnivores, are intermediate hosts. They get infected by oral uptake of the eggs of *E. multilocularis* (Eckert et al., 2001). Humans are aberrant intermediate hosts of *E. multilocularis*. Human alveolar echinococcosis (AE), caused by the larval stage of the small fox tapeworm *Echinococcus multilocularis*, is a zoonotic infection with an incubation period of 10 years in average (Ammann, 1992). AE is normally lethal if left untreated. In Germany, the incidence of reported human cases is small and approximately 25 cases per year, but a serious underreporting seems likely (Jorgensen et al., 2008). A total of 18,235 new cases of AE per year occur globally: 91% in China and 9% in other parts of the northern hemisphere (Torgerson et al., 2010). The parasite is distributed only in the northern hemisphere and definitive hosts are mainly fox species, the wolf, the coyote and the raccoon dog, according to their regional distribution (Vuitton et al., 2003). In Asia *E. multilocu-*

*laris* is widespread, covering large parts of Siberia, western and central parts of China and northern Japan (Gottstein, 2010). In Kyrgyzstan 64% of investigated red foxes tested positive for *E. multilocularis* (Ziadinov et al., 2010). Central China and north-east of Inner Mongolia are known as endemic areas since the 1980s (Vuitton et al., 2003).

In Europe new endemic areas of *E. multilocularis* have been identified in various countries such as Denmark (Saeed et al., 2006), Belgium (Vervaeke et al., 2005; Hanosset et al., 2008), the Netherlands (van der Giessen et al., 2004), Italy (Casulli et al., 2005), Lithuania (Šarkūnas et al., 2010) and Estonia (Moks et al., 2005) in the past decade in addition to the known endemic foci (Romig et al., 2006). The first occurrence of *E. multilocularis* in Sweden was reported only very recently, when a fox was found infected in the county of Västra Götaland in southern Sweden on 16 February 2011 (OIE<sup>1</sup>: 10263, 2011).

Results from investigations in Poland from 2005 and 2008 documented an increasing prevalence of the parasite in red foxes in the country except for its south-eastern part (Gawor and Malczewski, 2005; Ga-

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wor et al., 2008). Red foxes from Slovakia tested during the years 2000–2004 indicated high-endemic foci with prevalences of more than 30% in the northern and central part of the country (Miterpáková et al., 2006). A similar prevalence was detected in red foxes from Hungary in the northern mountain region bordering Slovakia in 2008 and 2009 (Casulli et al., 2010). Romig et al. (2006) reported an increasing density of infected host animals in Central Europe. The occurrence of *E. multilocularis* has also been reported in urban foxes from several European cities (Deplazes et al., 2004).

In Germany, the epidemiological situation concerning *E. multilocularis* has considerably changed in recent years. As shown by Berke et al. (2008), the average prevalence of the parasite in red foxes in Lower Saxony, northern Germany increased from 12% to 20% between 1991 and 2005. A similar development took place in Saxony-Anhalt: the prevalence in red foxes increased significantly from 10% to 25% between 1998–2004 and 2005–2007 (Denzin et al., 2009). In Thuringia, the prevalence of *E. multilocularis* increased from 11.9% (95% confidence interval 9.9%–14.0%) in 1990 to 42.0% (39.1%–44.1%) in 2005 (Staubach et al., 2011). While the infection was present in foxes only in the northwestern parts of Thuringia in 1990, it had spread over the entire state by 2004.

Romig (2009) suggested that additional neozootic mammals such as the raccoon dog *Nyctereutes procyonoides* are now involved in the lifecycle of this parasite in Europe. The raccoon dog is a medium-sized canid, originally distributed in East-Asia and is now widespread in northern and eastern Europe (Kauhala and Saeki, 2004) and its presence in Germany has been documented since the early 1960s (Nowak, 1984). In Germany *E. multilocularis* was first described in two male raccoon dogs in the northern part of Brandenburg by Thiess et al. (2001). Infections of raccoon dogs with *E. multilocularis* were also found in Poland (Machnicka-Rowińska et al., 2002) and in Latvia (Bagrađe et al., 2008). In a preliminary study conducted in northern Brandenburg, seven raccoon dogs tested positive (Tackmann et al., 2005). To our knowledge, these are the only published reports on the detection of *E. multilocularis* in raccoon dogs from European countries.

In Brandenburg and Mecklenburg-Western Pomerania,

Germany the hunting bags of the raccoon dog - about 90% of the total German hunting bag - have shown a remarkable increase since 1995 and comparable high population densities can be assumed in these regions (Anonymous, 1997; Anonymous, 2000; Anonymous, 2008<sup>2</sup>). These established raccoon dog populations are considered as a further potential for *E. multilocularis* (Tackmann et al., 2003, 2005).

Therefore, we hypothesize in accord with Romig (2009) that the raccoon dog may in addition to the red fox increase the risk for humans to become exposed to *E. multilocularis*. The purpose of this study was to estimate the prevalence of *E. multilocularis* in raccoon dogs from northeastern Germany based on a resilient data base.

## 1 Materials and Methods

Between 1 January 2000 and 31 December 2008 raccoon dog carcasses were taken from the hunting bag of 161 municipalities across Brandenburg ( $n=1,252$ ). The majority of samples, 966 (77%), were obtained from five northern counties: Ostprignitz-Ruppin, Prignitz, Oberhavel, Uckermark and Havelland. Brandenburg is located in northeastern Germany and has a total size of 29,481 km<sup>2</sup> (Fig. 1). Data on each investigated raccoon dog included the date of hunting/death, hunting location (local municipality; county) and the result of the examination for *E. multilocularis* and were stored and managed in a SQL-Database. The records were linked to a geographic information system (GIS) using ArcGIS ArcView 9.3.1 software (ESRI, Redlands, CA, USA). Animals were hunted and tested all year.

Before necropsy, the animals were frozen at -80°C at least for one week to minimize the infection risk for laboratory staff (Eckert et al., 2001). This procedure was repeated with the removed small intestines before examination in the laboratory. The small intestines were investigated by the Intestinal Scraping Technique (IST) with modifications described by Tackmann et al. (2006). In brief, the small intestines were laid out in three portions of equal length according to their anatomical sequence: anterior, middle and posterior portion. The whole gut was opened longitudinally. Smear samples of the mucosa from the posterior portion of the small intestine were taken with three microscopic slides, which were then pressed on square polystyrene petri dishes

<sup>1</sup> OIE, 2011. Immediate notification report. Report reference, Ref OIE: 10263, Report Date: 61/02/2011, Country: Sweden. Printed on: Wed Feb 16 17:23 Paris; Madrid 2011.

<sup>2</sup> Anonymous, 2008. Hunting report of the federal state of Brandenburg for the hunting year 2006/07 including executive summaries of the years 2004/05 and 2005/06 (In German). Ed by Ministry of Environment, Health and Consumer Protection of the Federal State of Brandenburg, Germany

( $100 \times 100 \text{ mm}^2$ ). These samples were examined with a stereomicroscope at eightfold to fiftyfold magnification. This procedure was repeated three times, so that finally the mucosa of the posterior portion of the small intestine was analysed with nine slides. If these nine samples proved to be negative, another six slides were examined to rule out infection. All specimens of *E. multilocularis* were counted and attributed to four classes according to the WHO/OIE Manual on echinococcosis in humans and animals (Eckert et al. 2001):

(+: 1–5 ++: 6–50 +++: 51–1,000 ++++: > 1,000).

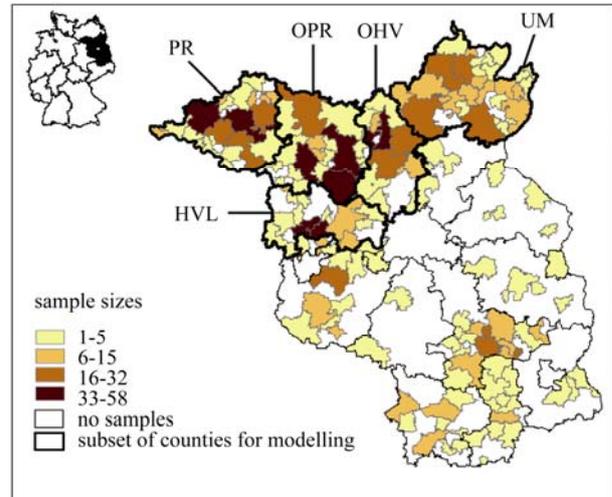
We estimated the raw prevalence for each municipality by calculating the ratio of positive cases relative to the total number of sampled raccoon dogs and summarized the data from 2000–2008 for the estimation of the prevalence over this time period.

Confidence intervals were determined using a calculator at [http://www.swogstat.org/stat/public/binomial\\_conf.htm](http://www.swogstat.org/stat/public/binomial_conf.htm). We assumed that the number of cases  $y_i$  in each spatial unit  $I$  is binomially distributed with  $y_i \sim \text{Binomial}(n_i, \pi_i)$ , where  $\pi_i$  represents the unknown true disease prevalence. We also assumed that  $n_i$  form a random sample of the animals inhabiting the respective area. Furthermore, the prevalence  $\pi_i$  of the municipalities  $i$  of Brandenburg is beta distributed with the parameters  $\alpha$  and  $\beta$ . We used a beta-binomial model to estimate the prevalence within each spatial unit. This probability model was fitted in a Bayesian framework, to our set of data capturing also unobserved quantities and heterogeneities within each municipality. We used the posterior mean of the true prevalence  $(\alpha + y_i) / (\alpha + \beta + n_i)$  to estimate the true disease prevalence (Gelman et al., 2000). In contrast to the beta-binomial model, the full Bayesian model would also incorporate the spatial structure of the data and captures heterogeneities between each municipality and time period. To this end, the underlying spatial and temporal smoothing parameters are also treated as unknown and have to be estimated from the data. Unfortunately models fitted with the available data set were not satisfying due to the high number of parameters and the low number of samples per space and time unit. We therefore decided not to use the non-fitting full Bayesian model.

Because of the limited sample sizes and spatial distribution of samples, but also because *E. multilocularis*-infected raccoon dogs remain undetected in southern counties, our analysis was restricted to data for five northern counties of Brandenburg: Ostprignitz-Ruppin (OPR), Prignitz (PR), Oberhavel (OHV), Uckermark (UM) and Havelland (HVL) (Fig.1).

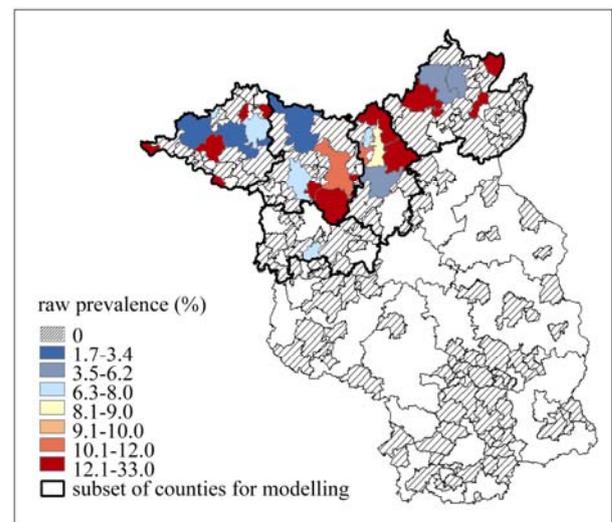
## 2 Results

Between 1 January 2000 and 31 December 2008, a total of 1,252 raccoon dogs originating from 161 municipalities in Brandenburg were examined for infection with *E. multilocularis*. All animals that tested positive for the parasite ( $n = 60$ : 4.79 %), were sampled in northern counties (Fig. 2). Within these counties, the sample sizes of different municipalities varied widely. There were municipalities where no raccoon dogs had been sampled.



**Fig. 1** Distribution of raccoon dogs investigated for *Echinococcus multilocularis* (2000–2008) in communities across Brandenburg, Germany

A subset of five counties for modelling is marked on the map: Ostprignitz-Ruppin (OPR), Prignitz (PR), Oberhavel (OHV), Uckermark (UM) and Havelland (HVL).



**Fig. 2** Calculated raw prevalence (%) for *Echinococcus multilocularis* in raccoon dogs in communities in northern Brandenburg, Germany from 2000 to 2008

The annual raw prevalence of *E. multilocularis* in raccoon dogs were calculated for five northern counties (Table 1).

At the scale of the municipalities, we calculated the raw estimated prevalences, based on summarized data for 2000 to 2008, which ranged from 1.7% to 33.0%, and found that major differences in prevalence existed, even between neighbouring municipalities (Fig. 2). In 62 of 91 municipalities of the subset of the above-mentioned counties, where samples were obtained, estimated prevalence was zero; these were mainly municipalities with small sample sizes.

To obtain reliable prevalence estimates for the infection of raccoon dogs with *E. multilocularis* in these five northern counties, we used a model based on the beta-binomial distribution (Staubach et al., 2002). According to model output, estimated prevalence ranged from 6.3% to 12.0% (Fig. 3). Two municipalities in the county of Prignitz (PR), situated in the west of the study region, showed the lowest estimated prevalences, plausible because sample sizes were high.

Focusing on the number of parasites, the examined intestines of infected raccoon dogs were sorted into four categories reflecting the intensity of infection (Fig. 4). The percentage of the category with the highest intensity (more than 1,000 specimens of *E. multilocularis*) was lower (4.7%), whereas the proportions of the other three categories were similar (26.6%–34.4%).

### 3 Discussion

Administrative structures are often the only feasible way to map samples in wildlife population surveys because available data are often aggregated at the level of administrative units and at regular time intervals. But when data are stratified the maximum likelihood estimates of the area-specific prevalence and its time-trend

can be seriously affected by random variation, in particular when the number of cases is small and the corresponding sample sizes are low in any single unit of space and time. Furthermore, the detection of spatial patterns is complicated by missing data in space and time as the complete coverage of all areas and time intervals is nearly impossible in wildlife surveys. Therefore, mapping of raw surveillance data in this manner may lead to false interpretations of disease clusters, disease-free areas and time-trends (Staubach et al., 2002).

Here, the sample sizes of the southern counties were too low to obtain reliable prevalence estimates by aggregating the data in time and space. We therefore had to focus our analysis on the five northern counties of Brandenburg, where sufficient data were available if the results were used in an aggregated form for a time period of nine years. As a consequence, analyzing data for each year separately to identify time-space patterns was not possible.

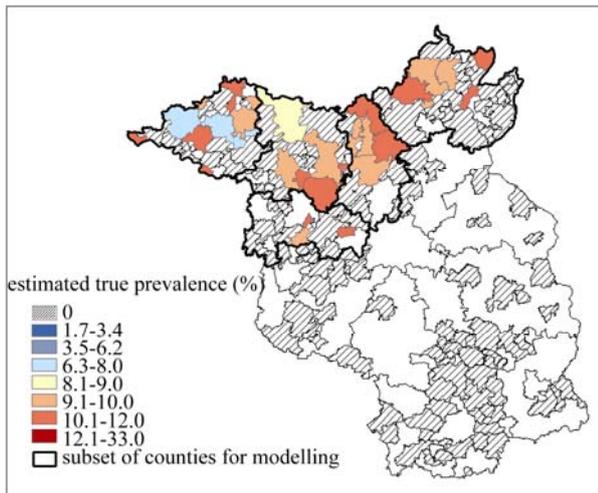
It remains undecided whether *E. multilocularis* infections occur in raccoon dogs in southern counties of Brandenburg. However, the presence of the parasite in these counties is expected since foxes are frequently found infected in these areas (FLI, Institute of Epidemiology, unpublished data). In regions where no samples were obtained, the presence of raccoon dogs can nevertheless be assumed, as demonstrated by local hunting bags (Anonymous, 2008).

In Brandenburg we detected 60 positive cases in five northern counties within eight years. Raw prevalence ranged from 2.6% to 10.7% in those years with higher sample sizes. By comparison, Machnicka-Rowińska et al. (2002) found two of 25 examined raccoon dogs infected in Poland, corresponding to a raw prevalence of 8%. In a survey conducted in Latvia, 12 of 57 raccoon

**Table 1** Raw prevalence of *Echinococcus multilocularis* in raccoon dogs in five counties of northern Brandenburg, Germany ( $n = 966$ )

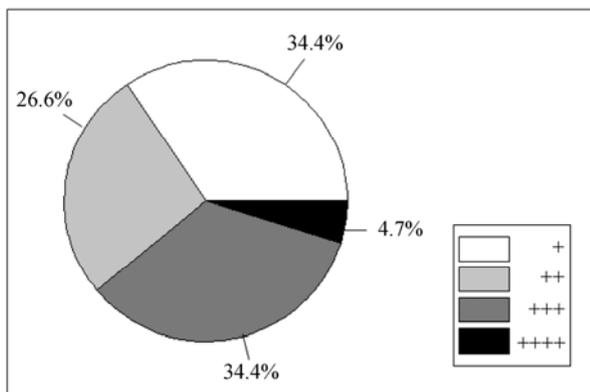
Year	Sample size	Positive cases	Prevalence (%)	95% confidence interval
2000	6	0	0	n.d.*
2001	4	0	0	n.d.
2002	5	1	20.00	0.51–71.64
2003	3	0	0	n.d.
2004	35	2	5.71	0.70–19.16
2005	253	27	10.67	7.15–15.15
2006	115	3	2.61	0.54–7.43
2007	239	10	4.18	2.02–7.56
2008	306	17	5.55	3.27–8.75

\* not determined



**Fig. 3** Estimated true prevalence (%) for *Echinococcus multilocularis* in raccoon dogs in communities in northern Brandenburg, Germany using a Bayesian beta-binomial model (Staubach et al., 2002)

Data are summarized for the period 2000–2008.



**Fig. 4** Intensity of infection with *Echinococcus multilocularis* in raccoon dogs *Nyctereutes procyonoides* in northern Brandenburg, Germany

$n = 60$ ; investigation period: 2000–2008. +: 1–5; ++: 6–50; +++: 51–1,000; ++++: > 1,000 specimens of *E. multilocularis*.

dogs (21%) tested positive (Bagrade et al., 2008). The study periods are unfortunately not mentioned in these publications.

At the municipality level, raw prevalence calculated for the northern counties varied in a wide range of 1.7%–33%, even between neighbouring municipalities. Presumably those differences were due to random variation and related to varying sample sizes. The applied beta-binomial model takes unequal sample sizes into account leading to a substantial reduction in variation (6.3%–12.0%), but there were still differences. For example, in two municipalities in the county of Prignitz (PR) the estimated prevalence was very low. However,

the results for these two municipalities seem reliable because large sample sizes were obtained. In the south-eastern part of the county of Ostprignitz-Ruppin (OPR), a true prevalence of 10%–12% was determined using the model. A former study in the counties PR and OPR showed two high endemic foci of infection with *E. multilocularis* in red foxes, one of them in the southeastern part of OPR (Tackmann et al., 1998). This result shows that *E. multilocularis* had been present in red foxes with an estimated prevalence of 23% since 1992 and may explain how the infection was transmitted to raccoon dogs in this area.

The life cycle of *E. multilocularis* is influenced by environmental conditions. Areas with humid conditions and low temperatures have been proposed as high risk areas for human exposure to eggs of the parasite (Hansen et al., 2004). In a Finish study raccoon dog preference for pastures, especially for damp meadows, is proposed (Kauhala and Auttila, 2010). Different environmental parameters among the investigated municipalities (e.g. percentage of wetlands) possibly influence the risk of infections with *E. multilocularis* in intermediate and definitive hosts (Staubach et al., 2001).

Focussing on the number of parasites found in infected raccoon dogs, we observed that infections with more than 1,000 specimens of *E. multilocularis* (category ++++) were less frequent than those of other categories of infection intensity. Thompson et al. (2006) found that worm burdens in foxes and raccoon dogs were similar after 35 days post inoculation (pi). After a time period of 63 days pi raccoon dogs harboured higher worm burdens than foxes. It was also noted that worms in intestines of raccoon dogs were significantly longer than in foxes. In experimental studies Kapel et al. (2006) estimated that the mean number of excreted eggs per parasite in raccoon dogs was 42 (95% CIs 19–85) and assumed a high biotic potential of the infections.

Because of its ability to colonize wide areas in a short period of time, based on a high reproduction rate (Kauhala and Saeki, 2004) and successful dispersal (Sutor 2008; Drygala et al. 2010), the raccoon dog will likely become of increasing importance as a definitive host for *E. multilocularis* in western Germany. The establishment of a new definitive host population may lead to changes in the temporal and spatial dynamics of infection with *E. multilocularis* in the affected regions.

In agricultural landscapes in Germany raccoon dog summer population densities (including offspring) are estimated at 3.4 individuals per square kilometre in Mecklenburg-Western Pomerania (Zoller, 2009) and 4.9

individuals per square kilometre in southern Brandenburg (Sutor and Schwarz, 2011). For the same study area Zoller (2009) calculated a red fox population density of 3.5 individuals per square kilometre. It can therefore be concluded that two definitive hosts for *E. multilocularis* live together in this area at a high density.

Home ranges between neighbouring raccoon dogs can overlap (Drygala et al., 2008) and overlapping of raccoon dog and fox home ranges of up to 93 % has been observed (Zoller, 2009). The use of common habitats by raccoon dogs and red foxes can increase the transmission of parasites (Kauhala and Holmala, 2006). Important intermediate hosts for *E. multilocularis* are small rodents such as *Microtus arvalis* and *Arvicola terrestris*, frequently distributed in agricultural landscapes across Central Europe. They represent one of the main food items in the diet of both red foxes and raccoon dogs (Sutor et al., 2010; Ansorge, 1991), leading to ideal conditions for the maintenance of the life cycle of *E. multilocularis*.

It remains unclear, however, whether the presence of the raccoon dog increases the prevalence of *E. multilocularis* and whether it raises the risk of AE in humans. Our future research will focus on the influence of host habitats and ecological factors on *E. multilocularis* infection and compare the definitive hosts the red fox and raccoon dog.

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