Parasitology International 89 (2022) 102583

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

# High variability in the number of *E. multilocularis* eggs in cat feces collected in the field

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ARTICLE INFO

*Keywords:*
*Echinococcus multilocularis*
Domestic cat
Biotic potential
Eggs
Zoonotic risk

ABSTRACT

*Echinococcus multilocularis* is the causative agent of alveolar echinococcosis that is considered as the most severe parasitic disease in Europe. The contribution of cat to environmental contamination by *E. multilocularis* is generally considered as extremely low based on results of experimental infections and worm burden estimations from natural infections. However, the recent collection of numerous cat feces from kitchen gardens in high endemic areas and the detection of *E. multilocularis* DNA in a significant number of these feces raise the question of the risk of human transmission from cats. This study aimed to provide a quantitative estimation of *E. multilocularis* eggs in feces from naturally infected cats. A field sampling conducted in 192 kitchen gardens during a joint study led to the collection and analysis of 597 cat feces, among them 7 (1.2%) yielded positive results for *E. multilocularis* real-time PCR. The entire pellets obtained after homogenization, filtration and centrifugation of a 5 *g*-sample for each of these 7 feces were examined under a stereoscopic microscope. After assessing their number, 20 taeniid eggs were individually isolated and specifically identified by real-time PCR. Morphologically mature *E. multilocularis* eggs were identified in 4 samples and the counting of 4 to 43 *E. multilocularis* eggs per gram in these samples, i.e. 62 to 2331 eggs per feces when the total mass of the feces is considered. The number of eggs counted in 2 feces suggests a biotic potential of some naturally infected cats that largely exceed the previous experimental estimations.

The cestode *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis that is considered as the most severe parasitic disease in Europe [1]. The lifecycle in Europe is essentially sylvatic and based on predation of small rodents by wild canids, mainly red fox (*Vulpes vulpes*). The rodent intermediate hosts are infected after ingestion of microscopic eggs, which are capable of surviving in the environment during months due to their resistant outer envelope [2]. After reaching the liver, the oncospheres will develop into metacestodes leading to production of protoscoleces in around two to four months. The viable protoscoleces are infectious for the definitive host when it ingests the parasitized prey. The intestinal development of protoscoleces into gravid worms with egg production will generally take between 28 and 35 days after infection. The total number of eggs excreted during one infection (i.e. biotic potential) is variable, notably according to host species [3,4]. A comparative study of experimental infection by *E. multilocularis* in definitive hosts described a 500-fold higher biotic potential for red foxes, raccoon dogs (*Nyctereutes procyonoides*) and dogs (*Canis familiaris*) than for domestic cats (*Felis catus*) [3]. Very low worm burden (3 to 30–50 worms) were generally found in the intestine of naturally infected cats [5,6]. A higher worm burden (7040 worms) was described by Umhang et al. [7], but consisted of only immature worms, and only a small proportion of them are assumed to reach the fully mature adults to release eggs. Both because of its low worm burdens and its excretion of a very small number of eggs of unproven infectivity, the cat is generally considered to play an insignificant role in the transmission of *E. multilocularis* [3]. However, the evidence of frequent consumption by cat of *E. multilocularis* intermediate host species [8], the detection of DNA of this parasite in many cat feces [7,9–13] and the high density of cat feces in kitchen gardens in endemic areas [14] raise concerns about the zoonotic risk associated to cat infections.

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https://doi.org/10.1016/j.parint.2022.102583
Received 17 January 2022; Received in revised form 2 April 2022; Accepted 2 April 2022
Available online 6 April 2022

Information on the biotic potential of naturally infected cats is needed to assess the epidemiological status of this host with respect to *E. multilocularis*.

The aim of this study was to estimate the total number of *E. multilocularis* eggs in feces of naturally infected cats from endemic areas. This quantitative estimation was then compared to that obtained from experimental infections to discuss the contribution of the cat to environmental contamination by *E. multilocularis*. A field sampling was conducted from January 2014 to December 2015 by Bastien et al. [15] in 192 kitchen gardens located in high endemic regions for *E. multilocularis* in northeastern France. It resulted to the collect of 1016 carnivore feces. Real-time coproPCR assays identified 597 of these feces as being from cats and detected *E. multilocularis* DNA in seven of them [9]. All the seven feces originated from different kitchen gardens. These feces were stored frozen since collection including one week at −80 °C for decontamination. Five grams were homogenized in 50 ml of distilled water and then filtered through a 120 μm nylon mesh. After centrifugation, the supernatant was discarded and the pellets completed again with 50 ml of distilled water before to be homogenized. These steps were repeated at least two times to clear the pellets. The entire pellets were then examined under a stereoscopic microscope (x200) and all the taeniid eggs observed during this examination were counted. When present, 20 taeniid eggs were individually isolated for each fecal sample in order to be submitted to individual DNA extraction (Nucleospin Tissue, Macherey-Nagel). A real-time PCR assay was performed to detect DNA of *E. multilocularis* with sequencing confirmation [16]. Five microliters of DNA were used in a final volume of 20 μl using Maxima Probe master mix and an internal control. All samples were tested in duplicate. The real-time PCR assays were realized using a Mx3005P thermocycler (Agilent) with an initial step of 95 °C for 10 min and 45 cycles of 95 °C for 15 s and 60 °C for 60s. When a Ct value was obtained for at least one duplicate, sequencing was realized from the PCR products to confirm identification of *E. multilocularis*. If no DNA of *E. multilocularis* was detected, the DNA samples were submitted to an endpoint PCR using primer Cest4-Cest5 in order to identify the Taenidae species involved after sequencing [17]. In absence of detection for the two PCR assays (*E. multilocularis* or Taenidae species), the eggs were excluded for the calculation of the number of eggs per gram (epg). We used the ratio of *E. multilocularis* eggs versus other Taenidae species obtained from the successful amplification among the 20 eggs isolated to estimate the number of *E. multilocularis* eggs per gram and calculate the total number of these eggs in the cat's feces.

No taeniid eggs were observed after filtration for 2/7 fecal samples from which *E. multilocularis* DNA was detected. Nevertheless, *Toxocora* sp. eggs were present for these two fecal samples confirming a correct flotation. Identification of *E. multilocularis* eggs was obtained for 4/5 samples with taeniid eggs, including one with coinfection by *Hydatigera taeniaeformis*. In the only fecal sample free of *E. multilocularis* eggs among these five samples, taeniid eggs were all corresponding to the cat tapeworm *Hydatigera taeniaeformis* (Table 1). The lowest average Cq values from fecal samples were obtained from the four samples with *E. multilocularis* eggs (30.6 to 35.8) compared to the three without eggs (36.9 to 39.2). The microscopic observation revealed morphologically mature *E. multilocularis* eggs with the presence of embryophores surrounding the oncosphere in the four cases. When present, the number of *E. multilocularis* eggs per gram (epg) was ranging from 4 to 43. Considering the total mass of each fecal sample, 62 to 2331 *E. multilocularis* eggs per fecal sample were estimated.

Our finding of a slightly lower amount of DNA in feces without eggs than in feces with eggs confirms that a substantial quantity of the copro-DNA from the parasite may not originated from eggs but rather from worm tissue cells. In addition, parasitic DNA detected in definitive host (i.e. cat) feces can also come from ingestion of infected prey if the feces were produced within a few days of that ingestion as it has been demonstrated for *Toxoplasma gondii*, [18]. Worm tissues or parasitic DNA from infected prey can explain alone the detection of

**Table 1**
Information and coprologic results of the seven cat feces with detection of *E. multilocularis* DNA by copro real-time PCR.

| Sample ID | Village | Kitchen garden ID | Collection date | Total weight of the fecal sample (g) | Average Cq values of *E. multilocularis* qPCR | Presence of taenid eggs | Proportion of *E. multilocularis* among taenid eggs | Number of *E. multilocularis* eggs/g | Total number of *E. multilocularis* eggs/ fecal sample | Proportion of *H. taeniaeformis* among taenid eggs | Number of *H. taeniaeformis* eggs/g |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 10,426 | Faxe | FAX08 | 07–11–2014 | 54.2 | 30.6 | yes | 18/20 | 43 | 2331 | 0/20 | 0 |
| 10,471 | Grandpré | GRA07 | 23–01–2015 | 36.3 | 35.5 | yes | 6/20 | 33 | 1198 | 2/20 | 22 |
| 10,371 | Lorry-mardigny | LOR09 | 18-12-2014 | 8.4 | 34.8 | yes | 15/20 | 14 | 118 | 0/20 | 0 |
| 10,459 | Boult-aux Bois | BAB13 | 21–01–2015 | 15.4 | 35.8 | yes | 15/20 | 4 | 62 | 0/20 | 0 |
| 10,550 | Avricourt | AVR12 | 10-03-2015 | 28.6 | 36.9 | yes | 0/20 | 0 | 0 | 15/20 | 10 |
| 10,390 | Avricourt | AVR08 | 18-12-2014 | 52.5 | 37 | no | / | / | / | / | / |
| 11,885 | Faxe | FAX08 | 16-12-2015 | 42.4 | 39,2* | no | / | / | / | / | / |

* Cq value obtained for only one duplicate.

*E. multilocularis* in the three feces where no eggs were observed. Regarding feces with *E. multilocularis* eggs, the DNA from these eggs comes in addition to the worm tissue source facilitating the molecular detection. A significant difference between distribution of Cq values from fox feces with or without eggs was also previously reported by Da Silva et al. [19].

Based on an experimental infection with 20,000 protoscoleces, the mean biotic potential of cats was estimated of 573 eggs excreted during an entire patent period estimated at 13 days in cats [3]. However, a highest daily eggs excretion of 856 eggs at 28 dpi was described for one cat in the same study. By comparison, the estimation of the number of eggs excreted in two fecal samples from our field sampling is 2 to 4-fold higher than this estimated biotic potential. The availability of only one fecal sample for each infection prevent us to assess duration of the patent periods in these natural infections, as well as the total number of eggs excreted in the course of the whole patent period. However, one can speculate that in each infection related to these two cases, several others feces from the same cats during the patent period will also containing eggs, potentially even more than described in the analyzed feces. Therefore, our data suggest that the biotic potential of some cats may be largely higher than expected even if the null or low eggs production associated with the three others positive fecal samples are in accordance to the very low biotic potential generally described for the species. The estimation of 440 eggs per gram reported for a cat from Hokkaido Island, Japan [20] which is largely (i.e. 10 fold) exceeding our highest estimations argues to excretion of large quantities of *E. multilocularis* eggs by cats from natural infections not being a very rare event. The high excretion of eggs by some cats may explain the detection of *E. multilocularis* DNA from soil samples of kitchen gardens where no fox feces were observed, including fenced kitchen gardens [9].

In a general way, natural infection in cats by *E. multilocularis* is generally restricted to a molecular diagnostic, and the number of eggs is rarely estimated when the presence of *E. multilocularis* eggs is confirmed resulting to scarce data related to the biotic potential. The data obtained from experimental infections are very informative regarding the global epidemiological situations but may underlooked some specific situations. Combination of different cat species, quantities of protoscoleces, *E. multilocularis* strains, ecological contexts and immunological expositions will results to a large variety of possibilities regarding the success of natural infections. The strong predation of rodents by cats can notably increase the chance of repeated infections and raise the need to assess the potential development of an immunity decreasing the worm burden and patent period, as described for foxes and dogs [21,22]. It also has to be noted that the high number of eggs excreted by infected cats in this study remains very inferior to the biotic potential of canids, as notably red foxes and dogs can excrete 279,910 and 346,473 eggs per individual, respectively [3]. Furthermore, the existence of a zoonotic risk associated to *E. multilocularis* eggs excreted via cat feces is currently not confirmed. Eggs obtained from feces of a cat experimentally infected by a North American strain of *E. multilocularis* has developed to metacestode after ingestion by a brown lemming [23] but those obtained after experimental infection of cats by a European strain has not been successful to infect mice, unlike those obtained from foxes, dogs and raccoon dog feces [3].

In conclusion, our data show that some cat feces may contain high numbers of *E. multilocularis* eggs, although the overall contribution of cats to environmental contamination by this parasite is probably extremely low compared to that of foxes. The presence of high numbers of *E. multilocularis* eggs in some cat feces reinforce the need to test the infectivity of these eggs obtained in natural conditions, which has not yet been reported. Specific protocols coupling rapid molecular diagnostic from decontaminated part of the cat fecal sample potentially followed by isolation of taeniid eggs are required. In absence of current in vitro test for evaluation of the infectivity, in vivo assays in rodents remains the only way to confirm the infectivity, and then a zoonotic potential.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Declaration of Competing Interest

The authors declare no competing interests in association with this study.

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