Observations regarding in vitro hatching of *Raillietina spp*. (Cestoda: Cyclophyllidea) onchosphere

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Abstract

The study has been realised in May 2016 having as purpose the observation of the onchosphere hatching process. The eggs of Raillietina spp. were aquired from feces belonging to Great Spotted Woodpecker (Dendrocopos major) that was naturaly infested. Initally, the samples were examined from a parasitological point of view using the Willis method. The egg concentrate has been obtained using the protocol described by VOGE and all. (1961) with some adjustments. The egg suspension was incubated at 30 °C for an hour. The hatching process of the oncosphere has been observed on the slide with the optical microscope (Motic B series) fitted with a Moticam 1000 camera, using the x400 magnification. Measuring has been realised with Motic Images Plus 2.0 software. The temperature of the slide in the moment of examination was 32°C. The whole hatching proces lasted 5 hours and went through the following stages: after half an hour the hexachant embryo has broken the basal membrane and entered the vitelline layer, the onchosphere was vigorously pushing and scraping the granular structure belonging to the vitelline layer, then the vitelline membrane and the egg shell was perforated after four and a half hours, thus completing the actual hatching process. After hatching, the onchosphere engaged in a series of swim-like motions in the liquid mass.

Keywords: *Raillietina spp., hatching oncosphere, great spotted woodpecker.*

Introduction

The hatching in cyclophyllidean cestodes is defined as the release of the oncosphere by the layers of the egg and its stimulation to activity (life) [8]. The hexachant embryo *in vitro* hatching was observed through the use of proteolitic enzymes (artificial gastric fluid and artificial intestinal fluid) [1, 4, 7], biological extracts from invertebrates in different environments [1, 5, 9] and by increasing presure to the eggs [6].

Material and method

The present study has been conducted in May 2016. The cestodes eggs were obtained from great spotted woodpecker (*Dendrocopos major*), naturally infected with *Raillietina spp*. The great spotted woodpecker is a common bird in the deciduous and coniferous forests but also in gardens and parks. It feeds on insect larvae found on trees but also with spruce seeds, or other cereals, and in spring it eats vegetable sap [3]. The fresh feces samples were colected in sterile polyethylene containers and were maintained at refrigeration temperature for 24 hours. Then they were examined using the Willis method.

The preparation of the egg concentrate was performed after the method described by VOGE et al. (1961), with the following modifications: commercial solution of NaCl 0,9% (pH = 5.5) was used for suspension washing.

Experimental protocol: The egg concentrate was agitatted and transfered to 10 Eppendorf tubes of 1 ml and maintained at a refrigeration temperature until examination (+4°C). Then each tube was incubated for an hour at 30°C. One drop of suspension was then examined under the

microscope in order to observe the movement of the hooks and the hatching of the hexachant embryo.

The examination was made using the optical microscop (MoticB series), equiped with a Motic 1000 camera and using x400 magnification. The measurements were made with the Motic Image Plus 2.0 software. The temperature during the examination was 32°C.

Results

The hatching process (fig.1-5). The sequences that precced the releasing of the hexachant embryo of *Raillietina spp.* from all egg structures, starting from the intact egg that presents motility of embryonary hooks (fig. 1) are: (I) the hexachant embryo is breaking the basal membrane (fig. 2), then it leaves the central area of the egg and penetrates the exterior layer (vitelline layer), (II) the incresed activity of the onchosphere, that with the help of hooks pushes and grazes vigorously the granular structure of the vitelline layer and vitelline membrane, (III) then it perforates the egg shell (fig. 3), (IV) the true hatching of the hexachant embryo (fig. 4).

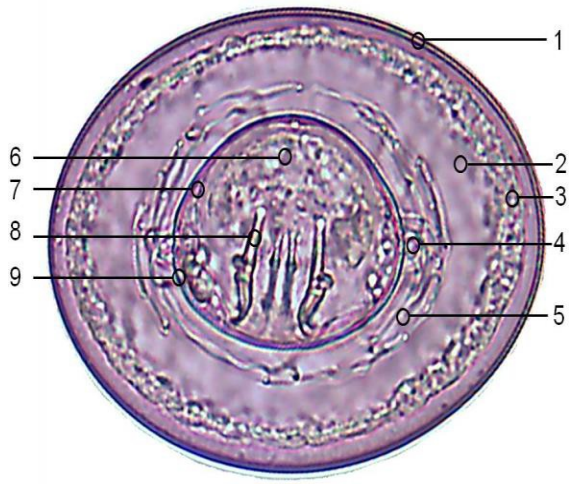


Fig. 1. Egg of *Raillietina spp.*
1 – Transparent shell; 2 – Vitelline layer/ Exterior layer;
3 – The granular structure of the vitelline layer; 4 – Polar thickenings (n=2);
5 – Filaments (appears from polar thickenings); 6 – Hexachant embryo (onchosphera);
7 – Onchosphere membrane; 8 – Hooks (n=3 pairs); 9 – Basal membrane.

After hatching, the onchosphere swims freely in the liquid mass (fig. 5). The entire hatching process was deployed in an 5 hour interval. Moreover, after approximatively 30 minutes the onchosphera releases from the central area of the egg by breaking the basal membrane and reaches the vitelline layer. Here, the hexachant embryo damages the other internal structures of the egg and after 4.5 hours the true hatching happens. Before the onchosphere enters the vitelline layer, the egg shell is smooth on both sides (internal and external).

Table 1. Morphometrics of *Raillietina spp.* eggs.

	L (µm)	W (µm)	T (µm)
Egg	103.73 ± 4.67	77.50 ± 5.36	-
Onchosphere	50.58 ± 1.26	40.04 ± 1.54	-
Hooks	20.86 ± 1.13	-	(D) 2.48 ± 0.27 (M) 4.30 ± 0.34 (P) 1.63 ±0.21

L – length, W– width, T – thickness, D – distal part, M – medial part, P – proximal part.

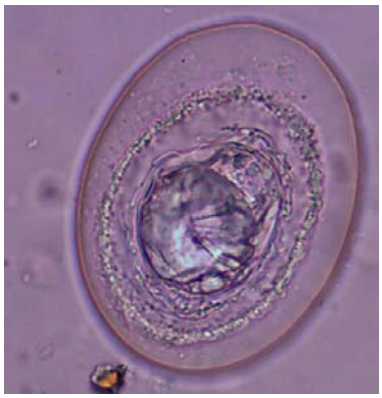


Fig. 2. First step of hatching: breaking the basal membrane

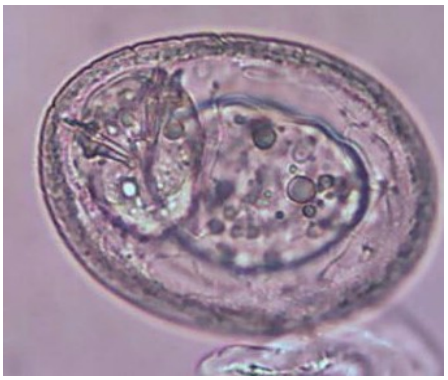


Fig. 3. The onchosphere pushes and grazes the internal structures of the egg

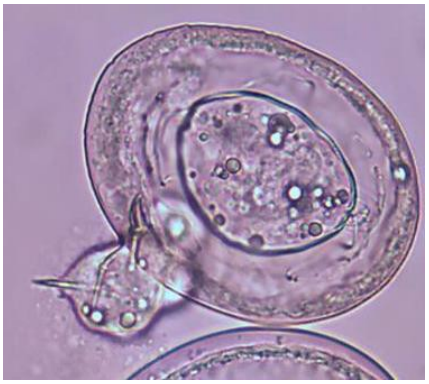
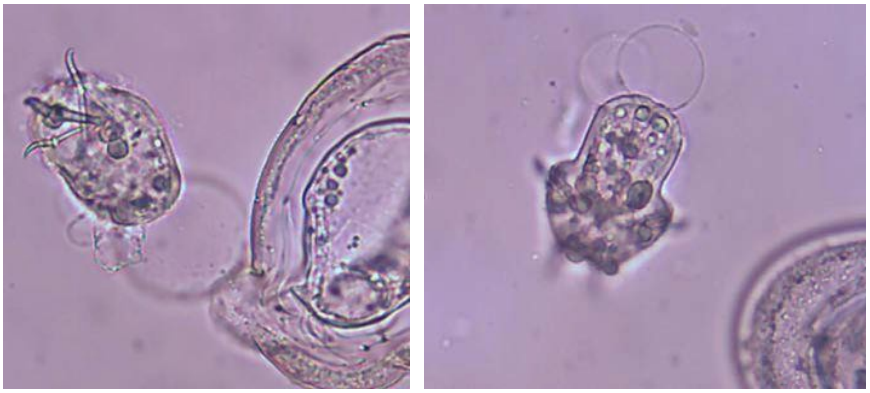


Fig. 4. The true hatching



(a) **(b)**

Fig. 5. Free and viable oncosphere

Discusions

The differences between the anatomic and histochimic structure of the cyclophyllidean eggs, may lead to different requirements of incubation fluid [8]. In the case of *Raillietina spp.* eggs, the hatching has been obtained without simulating the conditions found inside the intermediate host. The only physico-chemical stimulation applied to the egg shell was the osmotic shock produced by the saturated solution used in the process of obtaining the egg concentrate.

The effect of the Tyrode's extract of adults or larvae of *Dermestes vulpinus* (pH 7,2 to 7,8) produced the hatching of the onchospheres of *Hymenolepis diminuta* after 15 minutes to half an hour [9].

Conclusions

The in vitro hatching of the hexachant embryo from the eggs of *Raillietina spp.* mantained in an environment with pH 5.5 and temperature of 32ºC, was obtained in approx. 5 hours.

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