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Extremely Low Frequency Magnetic Field and the Hatching Rate of *Fasciola hepatica* Eggs, the Fecundity and Survival of Liver Fluke-infected Snail, *Lymnaea truncatula*

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Eggs of *Fasciola hepatica* were exposed for 10 days to extremely low frequency magnetic field (ELFMF) at the frequency of 50 Hz and density of 2 mT (rms). The results show an accelerated hatching of *F. hepatica* eggs in relation to control (non-exposed) group. The host snails, *Lymnaea truncatula*, were divided into three groups; those of groups I and II were infected with the miracidia of *F. hepatica* hatched from control egg culture, whereas those of group III were infected with miracidia hatched from eggs affected by ELFMF. Thereafter, snails of groups II and III were exposed to ELFMF for 53 days, whereas those of group I were not exposed. At day 14 post infection, a significant decrease was observed in the number of cocoons laid by snails of group III, compared with control. Also, significant mortality in group III snails was observed 42 days post infection. The increased mortality and a lower number of cocoons laid by group III snails have probably resulted from enhanced stimulation of metacercarial parthenogenetic reproduction in consequence of infecting the molluscs with miracidia reared under ELFMF.

Key words: Extremely low frequency magnetic field (ELFMF), eggs, *Fasciola hepatica, Lymnaea truncatula*.

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Despite the considerable progress in research on the effects of extremely low frequency magnetic fields (ELFMF) on living organisms, the outcomes of long-term impact have not yet been sufficiently studied. It has been demonstrated that early stages of embryonic development of various species are responsive to magnetic fields (CAMERON *et al*. 1993). KU NA-GRYGIEL *et al*. (2005) observed an accelerated rate of embryogenesis in *Ascaris suum* eggs incubated in a magnetic field and a much higher mortality rate of the larvae, whereas SKAULI *et al*. (2000) reported delayed hatching in zebra fish (*Danio rerio*) under a 50-Hz alternating magnetic field (AC). An increased intracellular concentration of calcium ions and acceleration of morphogenetic movements during amphibian gastrulation was noted under 50-Hz ELEMF (KOMAZAKI & TAKANO 2007). Delayed gastrulation as a result of 60-Hz ELMF was demonstrated in a sea urchin, *Strongylocentrotus purpuratus* (cf. ZIMMERMAN *et al*. 1990). Exposure of the eggs of a sea urchin, *Paracentrotus lividus*, to 75-Hz ELFEF resulted in a loss of synchronization of the first cell cycle, irregular separation of chromatids, and also in malformed embryos (RAVERA *et al*. 2006). The teratogenic effect of 50-Hz ELFMF on the embryogenesis of a parasitic nematode, *Ascaris suum*, was demonstrated in our previous report (KU NA-GRYGIEL *et al*. 2005). Developmental abnormalities were also found in chicken embryos as an effect of 50-Hz ELFMF (LAHIJANI & GHAFOORI 2000).

It has been confirmed that the principal parameters contributing to the effect of the electromagnetic field include the field parameters, conditions of the exposure, the species exposed, and the stage

of its ontogeny. A strong magnetic field (7 T) results in delayed egg hatching and a reduced hatching rate in tobacco budworm, *Heliothis virescens* (cf. PAN 1996). A similar effect was observed for mosquito eggs (PAN & LIU 2004). Altered furrow cleavage was noted in eggs of frogs of the genus *Xenopus* subjected to a strong static magnetic field (DENEGRE *et al*. 1998).

In general, the effects of ELFMF on invertebrates are poorly understood. We hope that the presently described experimental model (i.e., the *Fasciola hepatica–Lymnaea truncatula* parasite-host system), will be efficient in analysing the effects of ELFMF on hatching of the parasite and also on its further development in the host's organism exposed to ELFMF, as well as in relation to the response of only the host to ELFMF (reproduction, survival).

The liver fluke (*Fasciola hepatica*) is a cosmopolitan trematode found in the liver and bile ducts of ruminant mammals and humans. The eggs expelled by the host with faeces hatch in an aquatic environment into the first larval stage, the miracidium, which actively invades an intermediate host. The intermediate hosts are snails of the family Lymnaeidae, in Poland represented mainly by *Lymnaea truncatula* and *L. occulta*. Subsequent stages of the liver fluke develop in the snail: the sporocyst, the first and the second generation of rediae, and the cercariae, which leave the snail's organism and encyst to form metacercariae.

A number of abiotic (physical and chemical) as well as biotic factors influence both embryogenesis and larval development of *F. hepatica* in the snail (DREYFUSS *et al.* 1999; VIGNOLES *et al*. 2004). The success of *F. hepatica* infection in freshwater snails depends on many factors, including host age, water temperature, and food availability (ABROUS *et al.* 2001).

Pesticides retard embryogenesis and decrease hatching rate of *F. hepatica* (CHRISTIAN *et al*. 1985; BIELECKI 1985). Likewise, X-radiation slows down embryonic development and reduces the hatchability of miracidia, whereas focused laser light accelerates embryogenesis and increases hatching success (BIELECKI 1986).

The aim of this study was to evaluate the effects of extremely low frequency magnetic fields (ELFMF) on the hatchability of *Fasciola hepatica* eggs, the fecundity (the number of cocoons laid) and survival of *L. truncatula* snails infected with *F. hepatica* miracidia (exposed or unexposed to a ELFMF).

Material and Methods

F. hepatica eggs incubation

Adult liver flukes were obtained from the bile duct of rats experimentally infected with metacercariae of *F. hepatica*. Eggs of *F. hepatica* were collected from the uteri of adult flukes and were incubated in tap water at 25°C in 6 Petri dishes for 10 days. Three dishes containing eggs suspended in water were placed under ELFMF, while three others (unexposed) were the control. At temperature 22-26°C embryonic development of *F. hepatica* takes about 1-2 weeks (GARCIA 2007). Due to the fact that microscopic trial inspections carried out on day 6 of incubation showed the presence of approx. 95% motile miracidia in the culture exposed to ELFMF, the cultures were kept in darkness for the remaining period of embryogenesis.

On day 10 of incubation, 0.1 ml of medium from each culture (ELFMF exposed and control) was sampled and 100 randomly collected eggs from each sample were examined after previous exposure to light, which resulted in instantaneous simultaneous hatching of miracidia. Also, the numbers of unembryonated eggs, eggs containing miracidia (with closed operculum), and hatched eggs (with opened operculum) were determined.

Lymnaea truncatula culture

The snails *Lymnaea truncatula* were raised in the authors' own culture according to TAYLOR and MOZLEY (1948). The snails were fed *Oscillatoria* sp. cultured on soil medium collected in a natural body of water. Each snail (shell 4 mm in diameter) was exposed to contact infection with 10 miracidia for a period of 4 hours. The infected snails were divided into 3 groups, 8 specimens each. The snails of groups I and II were infected with the miracidia obtained from control cultures, whereas the snails of group III were infected with miracidia reared from eggs that had been incubated in ELFMF. After infection, snails of groups II and III were placed in ELFMF, while those of group I – outside ELFMF (Fig. 1).

The number of cocoons laid by *F. hepatica*-infected snails on day 14 post infection was assumed as an index of snail fecundity, whilst the survival rate of the snails was monitored on days 14, 21, 28, 33, 40, 42, and 53 post infection (dpi). Furthermore, the onset of metacercarial production was estimated in the *F. hepatica*-infected snails.

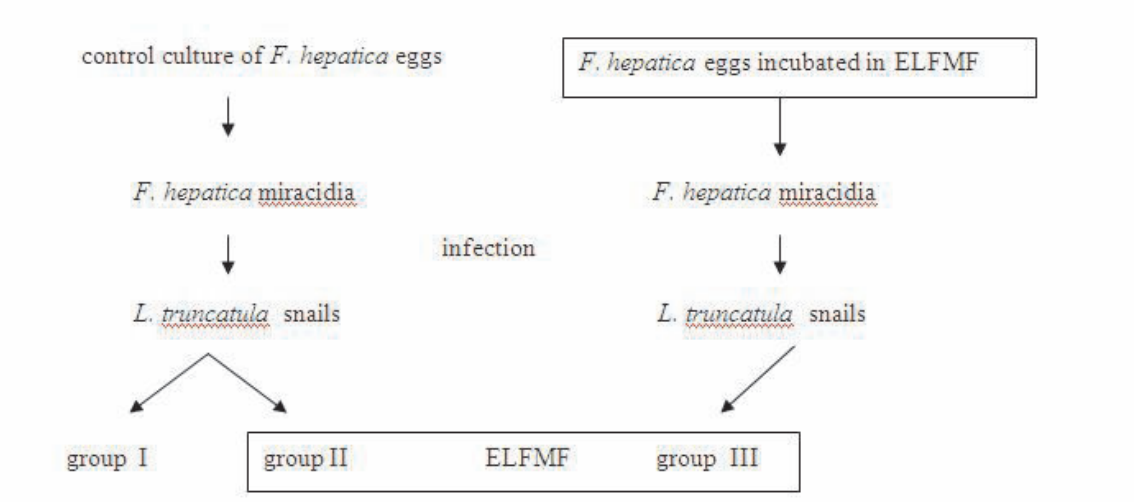

Fig. 1. Scheme of the experiment. Groups of snails: groups I and II infected with the miracidia obtained from control cultures, group III infected with the miracidia developed from eggs that had been incubated in ELFMF, groups II and III exposed to ELFMF, group I unexposed to ELFMF.

Magnetic field

The experimental cultures of *F. hepatica* eggs and *F. hepatica*-infected *L. truncatula* snails were placed under an extremely low-frequency 50 Hz magnetic field, produced by a pair of Helmholtz coils connected to a power network. The coils were 17 cm in diameter and 7 cm distant from each other, producing a magnetic field in a vertical direction. The intensity of the magnetic field was 2.0 mT (rms) with homogenous better than ±0.2 mT in the region Petri dishes within the coils. The field was checked using a Hall-effect probe magnetometer (teslometer TH-24; Aspan, Warsaw). The Helmholtz coils were mounted on a wooden frame surrounding a cork-jacketed cylinder into which the culture flasks were placed. The cork jacket isolated the cultures from heating by the coils. The difference in temperature in the experimental and control groups did not exceed 1.5°C.

Statistics

The comparisons were performed using two-sided Fisher's exact tests and chi-square tests.

Results

In the samples of eggs collected from the cultures exposed to ELFMF we found about 95% motile miracidia as early as on day 6 of incubation, while in the control culture only partly developed larvae could be observed at the same time.

On day 10 of incubation, the hatching rate of the eggs developing under ELFMF was more than two fold (2.4 times) higher compared with the control (Table 1). At the same time, the control cultures had approximately 3 times more eggs containing developed miracidia, though still not mature enough to hatch (Table 1). These differences were statistically significant; no significant differences, on the other hand, were found in the frequency of unembryonated eggs in both cultures.

Table 1

Number of *F. hepatica* eggs on day 10 of incubation: nonembryonated eggs, eggs before hatching, eggs after hatching in the control culture and the culture exposed to ELFMF

Eggs	Control n = 300	ELFMF n = 300
nonembryonated	32	44
before hatching	187	61*
after hatching	81	195*

* – significantly different from control group (chi-square test, $P<0.001$).

The snails of groups II and III laid fewer cocoons on day 14 post infection (pi) compared with the control; significant differences, however, were observed in group III only (Fig. 2).

From day 28 pi on, a gradual decrease in the number of infected snails was recorded in groups

II and III. A statistically significant drop (2.7-fold) in the number of infected snails was found on day 42 pi in group III, as compared with control (Fig. 3). On day 53 pi, live *L. truncatula* snails were found only in groups I and II.

The first metacercariae were observed on day 40 pi in the cultures of snails of group III, and two days later in groups I and II (42 dpi).

Discussion

Our observations have revealed that ELFMF accelerated both egg hatching and further larval development of *F. hepatica* in the mollusc hosts. These findings are consistent with the results of our previous studies, which have also demonstrated a stimulating effect of ELFMF on embryonic development rate in the nematode *Ascaris suum* (cf. KU NA-GRYGIEL *et al*. 2005).

Accelerated hatching of miracidia from the eggs incubated under ELFMF is probably due to the fact that ELFMF stimulates both protein biosynthesis and cell division (BLANK & GOODMAN 1997). TOMAN *et al*. (2002) reported a higher hatching rate in ELFMF-exposed (0.07 T 20-40 min) eggs of Hampshire chickens. Under SVMF (sinusoidal varying magnetic fields), increased proliferation of chicken embryonic fibroblasts was observed by KATSIR and PAROLA (1998). According to WOLF *et al*. (2005) a 50 Hz ELFMF results in 30% increase in cancerous cells proliferation (HL-60 leukaemia cells) as well as fibroblasts in rats.

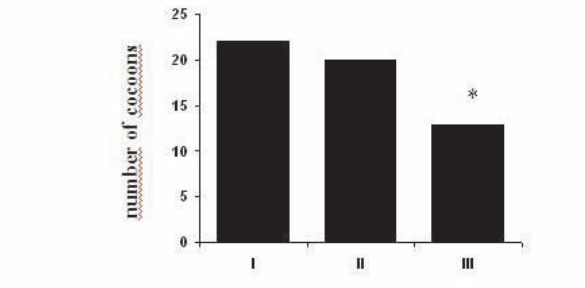

Fig. 2. Number of cocoons laid by *L. truncatula* snails infected with *F. hepatica* miracidia at 14 dpi in groups I, II and III. Group III* – significantly different from control group I ($p<0.003$), chi square test with Yates correction factor.

There are contrasting results, however, in terms of cell proliferation reported by other authors who studied the effects of ELFMF. CAMERON *et al*. (1993) reported an inhibitive effect of ELFMF on sea-urchin embryonic proliferation, while TOFANI *et al*. (2002) observed a similar effect on cancer cell division in mice. On the other hand, no changes in cancer cell proliferation rates were found under extremely low-frequency magnetic fields at 60Hz (2-500 μT) (YOSHIZAWA *et al*. 2002).

In the light of both our observations and those described by other authors, we can presume that the biological effects of magnetic fields depend on many factors, such as radiation dose or different susceptibility of individual organisms.

The considerable decrease in the number of *F.hepatica*-infected group III snails at 42 dpi, as compared with the control, may have resulted from the impact of ELFMF exerted both on *F. hepatica* during embryogenesis and on the intermediate host.

The miracidia obtained from ELFMF-exposed *F. hepatica* eggs probably exhibited more intensive parthenogenetic reproduction, which could have led to increased mortality of the snails. Parthenogenetic stimulation of metacercarial production under ELFMF may be reflected also in the earlier metacercarial production observed in the snails of group III.

An increased level of asexual reproduction and regeneration was observed in a turbellarian, *Dugesia tigrina*, under weak static (DC) and alternating (AC) magnetic fields (NOVIKOV *et al*. 2002, 2008).

Some parasites, including *F. hepatica* in the snail, are known to lead to impairment of the reproductive system of the host organism, referred to as parasitic castration (GRACZYK & FRIED 1999). The drop in the number of cocoons laid by the

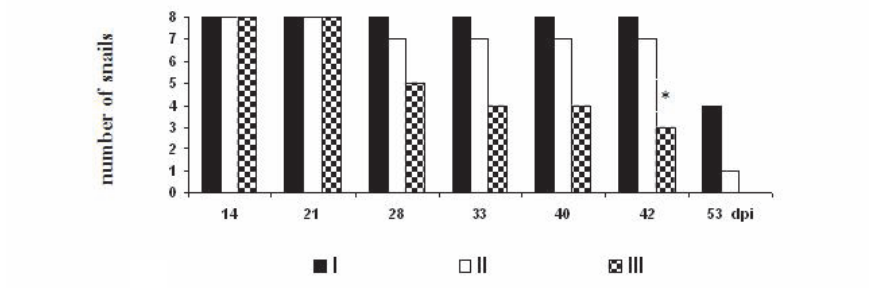

Fig. 3. Number of *L. truncatula* snails infected by *F. hepatica* between 14 and 53 dpi. I, II, III – groups of *F. hepatica*-infected snails Group III* – significantly different from control group I (P ~0.03), two sided Fisher's exact test.

snails observed in our experiment, especially in group III (at 14 dpi), may indirectly confirm the stimulating effect of ELFMF on *F. hepatica* larval development in the mollusc organism and may lead to a reduced number of infected snails. On the other hand, we cannot rule out an inhibiting effect of ELFMF on the fecundity of the snails. It has been demonstrated that electromagnetic nano impulse exposure results in reduced fertility of *Caenorhabditis elegans* (cf. BOJJAWAR *et al*. 2006).

It is evident from this report that low intensity 50 Hz ELFMF results in an accelerated hatching of *F. hepatica* eggs. ELFMF exposure in the parasite–host system of *F. hepatica–L. truncatula* is manifested in increased mortality and reduced fecundity of the host organism infected with the liver fluke, the entire embryogenesis of which was also affected by ELFMF. It can be concluded that ELFMF is not indifferent for the development of *F. hepatica* or its intermediate host.

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