

Short Communication

The Effect of an Extremely Low Frequency Magnetic Field on Larvae Production in the Parasite-Host System: *Fasciola hepatica-Galba truncatula*: a Preliminary Study

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The aim of this study was to determine the effect of an extremely low-frequency magnetic field (ELFMF) on the production of liver fluke larvae in a parasite-host system: *Fasciola hepatica* – *Galba truncatula*. Both *F. hepatica* eggs and *F. hepatica*-infected snails were exposed to an ELFMF (50 Hz, 2.0 mT) for 14 days and 36 days, respectively. *F. hepatica*-infected snails were divided into 4 groups, 10 specimens each. The snails of groups I and II were infected with *F. hepatica* larvae – miracidia obtained from control cultures, while the snails of groups III and IV were infected with miracidia reared from eggs that had been incubated in an ELFMF. After infection, the snails of groups II and IV were placed in an ELFMF, while those of groups I (control) and III were housed outside the ELFMF. At 36 days post-infection (dpi) there were no statistically significant differences between the number of *F. hepatica* larvae – cercariae and metacercariae, obtained from *G. truncatula* snails in the control group (group I) and the snail groups exposed to ELFMF (groups II, III and IV). However, a statistically significant difference between the average number of *F. hepatica* larvae in snail groups III and IV may indicate that the duration of exposure to ELFMF, i.e. embryogenesis period vs. the entire larval development, played a role in the production of *F. hepatica* larvae, and resulted in a reduction of their number.

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

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The biological effects and mechanisms of the influence of magnetic fields on living organisms are not yet fully understood. It has been demonstrated that embryonated organisms are more susceptible to magnetic fields than mature organisms (KUŻNA-GRYGIEL *et al.* 2005). The early stages of embryonic development of various species are responsive to magnetic fields (CAMERON *et al.* 1993; KOMAZAKI & TAKANO 2007; GONET *et al.* 2009). An accelerated rate of embryogenesis was

observed in parasitic nematode (*Ascaris suum*) eggs incubated in an extremely low-frequency magnetic field (ELFMF) (KUŻNA-GRYGIEL *et al.* 2005). In our previous studies, accelerated hatching of larvae (miracidia) under the influence of ELFMF was also found in the liver fluke (*Fasciola hepatica*) (KOŁODZIEJCZYK *et al.* 2010).

The life cycle of *F. hepatica* involves an intermediate host (snail) and a definitive host (herbivores and humans) in whom mature flukes

parasitize the liver and bile ducts. The life cycle of *F. hepatica* includes various stages: egg, miracidium, sporocyst, redia, cercaria, metacercaria and adult stages. Adult flukes in the bile ducts of the mammalian host produce eggs that are expelled. In an aqueous environment the eggs become embryonated and miracidia hatch. The miracidia penetrate a suitable snail host (*Galba truncatula*) within 8 h after hatching. In the snail, the parasite undergoes several developmental stages: sporocysts, rediae and cercariae, to emerge from the snail and become free-swimming. Generally cercariae leave the snail 4-7 weeks after infection. The cercariae encyst as metacercariae on aquatic vegetation or other surfaces after a few minutes to 2h after emergence. After ingestion by the definitive host, metacercariae excyst in the duodenum and migrate to the bile ducts, where the flukes reach sexual maturity (ANDREWS 1999).

The aim of this study was to determine the potential impact of an ELFMF on different stages of *F. hepatica* (i.e. during embryogenesis and/or during larval development in the snail).

Adult liver flukes were obtained from the bile ducts of naturally infected cattle. Eggs of *F. hepatica* were collected from the uteri of adult flukes (CHRISTIAN *et al.* 1985) and were incubated in tap water at 24°C in two Petri dishes for 14 days. One dish containing a suspension with eggs was placed under an ELFMF, while the other was kept away from the ELFMF as a control. The cultures were kept in darkness for the period of embryogenesis. On day 15 of incubation, after exposing the eggs from the two cultures to light for 1h, the resultant miracidia were used for the infection of the snails.

The snails of *Galba truncatula* (n=40) were raised in the authors' own culture according to TAYLOR & MOZLEY (1948). The snails were fed *Oscillatoria* sp. algae cultured on soil medium collected in a natural body of water. The procedure of snail culture was described earlier (KOŁODZIEJCZYK *et al.* 2015). The snails (measuring 7-8 mm

in shell height) were divided into 4 groups of 10 specimens each. Snails of groups I (control group) and II were infected with the miracidia obtained from control cultures (non-exposed to ELFMF), whereas snails of groups III and IV were infected with miracidia reared from eggs that had been incubated in the ELFMF. Each snail (from groups I-IV) was exposed to 5 newly hatched miracidia for a period of 4 h. After infection, the snails of groups II and IV were placed in the ELFMF, while those of groups I and III remained outside the ELFMF (Fig. 1). Snails (from groups I-IV) were placed in four separate beakers (n=10) and kept at 24°C for 36 days. The cultures were observed every 3-4 days. At 35 dpi and 36 dpi, after moving the snails to a different vessel, we determined the number of metacercariae encysted on the walls of the beakers where the snails were kept. All snails from groups I-IV survived until 36 dpi and on that day they were crushed to determine *F. hepatica* infection. Each snail was crushed in a separate vessel. The assessment of the number of larvae (cercariae and metacercariae) obtained from each subject was performed under a stereomicroscope at least 4 h after dissection.

The experimental cultures of *F. hepatica* eggs and *F. hepatica*-infected *G. truncatula* snails were placed under a 50 Hz sinusoidal magnetic field produced by a solenoid connected to a power network. The solenoids were 24 cm in diameter and 16 cm long and produced a magnetic field in a vertical direction. The intensity (induction) of the magnetic field was $B = 2.0$ mT, (rms, root mean square), therefore amplitude $B_{\max} = \pm \sqrt{2} B$ with a homogenous field exceeded ± 0.2 mT in the region of the Petri dishes, as measured with a Hall-effect magnetometer probe (teslometer TH-24; Aspan, Warsaw). The solenoids were mounted on a wooden frame surrounding a cork-jacketed cylinder into which the crystallizers containing eggs or snails were placed. The control eggs and control snails were placed in a "non active" solenoid, in

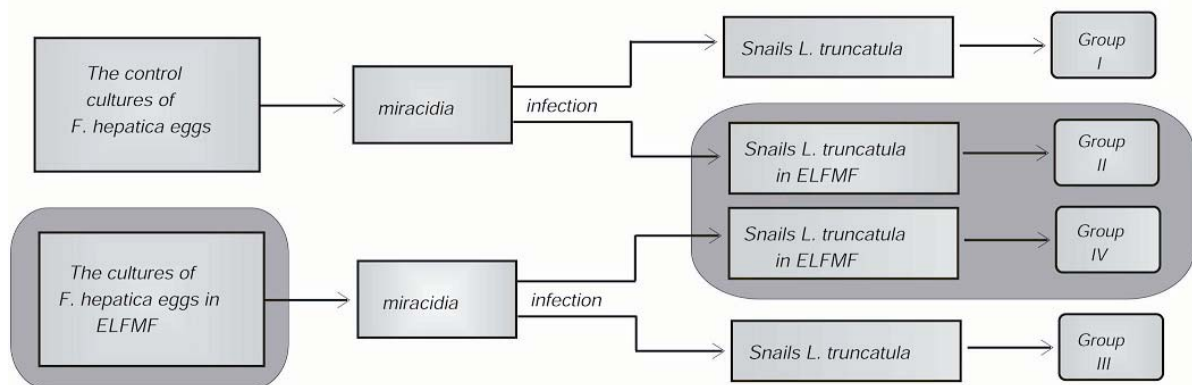


Fig. 1. Schema of the experiment. The oval dark grey areas represent ELFMF exposure (*F. hepatica* eggs and *F. hepatica* infected snails).

which the wire was spooled in a specific way, i.e. each wire loop had the corresponding loop with current flow in the opposite direction. An additional resistance adjusted the current so that it was the same as in the active solenoid. This setup resulted in the total attenuation of the magnetic field. The thermal effect was the same in both experimental and control probes.

Relevant numerical data are presented as means with 95% confidence intervals. The Shapiro-Wilk test revealed normal data distribution. Statistical analyses were performed using ANOVA (Statistica, StatSoft, Inc. Tulsa, OK 74104, USA). A Tukey honest significant difference (HSD) test was applied for pairwise comparisons (Statistica, StatSoft, Inc. Tulsa, OK 74104, USA). Results were considered to be statistically significant at $P < 0.05$.

The presence of the first *F. hepatica* metacercariae on the dish walls was observed in cultures of snails at 35 dpi in groups II, III and IV, in contrast to the control group (group I) where none were observed. At 35 dpi the largest number of metacercariae was found in group III (670), with about 3 times fewer metacercariae in group II (231), and the least in group IV (11). After the next 24 hours, at 36 dpi, the presence of metacercariae on the dish walls was also observed in the control group (I). At 36 dpi, after removal of the snails from the beakers, the total number of metacercariae on the walls of the beakers in individual groups showed a similar regularity – the highest number of metacercariae was found in group III (211), followed by group II (133), group IV (56) and the lowest number (34) in the control group. These data may indicate that exposure of *F. hepatica* to ELFMF, both during embryogenesis and/or during the further development of larvae in snails (groups II, III and IV), accelerated the production of metacercariae, albeit to varying degrees. In our previous study (KOŁODZIEJCZYK *et al.* 2010), we observed that on the 10th day of incubation, the hatching rate of *F. hepatica* eggs developing under ELFMF was more than twofold higher compared to the control. In the statistical analysis, we did not take into account the number of metacercariae obtained before the dissection of snails, i.e. at 35 and 36 dpi, since the data concerned entire groups of snails and not individual specimens. The mean numbers of larvae (cercariae and metacercariae) per snail obtained by dissection at 36 dpi in each snail group are shown in Figure 2. No statistically significant difference in the number of larvae of *F. hepatica* was found between the control group and the other groups of snails (II, III and IV). Statistically significant differences were found only between groups III and IV. These results may indicate that exposure of *F. hepatica* to ELFMF during embryogenesis and/or further life cycle stages in the

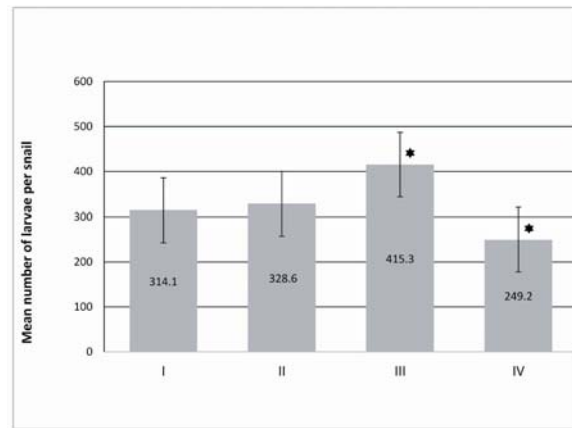


Fig. 2. The average number of *F. hepatica* larvae (cercariae and metacercariae) in the groups of infected snails at 36 dpi. – significant difference between groups III and IV ($P = 0.011$).

host did not affect the production of liver fluke larvae at 36 dpi. However, a statistically significant difference in the average number of larvae of *F. hepatica* between snail groups III and IV may indicate that the duration of exposure to ELFMF (i.e. embryogenesis period – 14 days vs. the entire larval development period – 50 days), played a role in the production of *F. hepatica* larvae, and resulted in a reduction of their number. We are not aware of any previous studies concerning the effect of ELFMF on the production of trematode larvae.

In conclusion, our findings do not exclude the stimulatory effect of ELFMF on the production of *F. hepatica* larvae (especially during embryogenesis – group III), because cercarial shedding is a dynamic process that can start as early as 27 days post-infection (LEE *et al.* 1995) and ends with the death of the snail. Our preliminary research concerned the production of larvae at a specific period (36 dpi) in the infected snails. Future research should take into account not only the number of larvae obtained during the dissection of the host, but also in the period before the dissection.

References

- ANDREWS S.J. 1999. The life cycle of *Fasciola hepatica*. (In: Fasciolosis. J.P. Dalton ed. CABI Publishing, Wallingford): 1-29.
- CAMERON I.L., HARDMAN W.E., WINTERS W.D., ZIMMERMAN S., ZIMMERMAN A.M. 1993. Environmental magnetic fields: Influences on early embryogenesis. *J. Cell. Biochem.* **51**: 417-425. DOI: 10.1002/jcb.2400510406.
- CHRISTIAN F.A., TATE T.M., TESFAMICHAEL T., LEBLANC C. 1985. Effects of MSMA (Monosodium Methanearsonate) and Diuron on embryo development and hatching of *Fasciola hepatica* miracidia. *Environ. Pollut. (Series A)* **38**: 1-7.
- GONET G., KOSIK-BOGACKA D.I., KUŻNA-GRYGIEL W. 2009. Effects of extremely low-frequency magnetic fields on the oviposition of *Drosophila melanogaster* over three generations. *Bioelectromagnetics* **30**: 687-689.

- KOŁODZIEJCZYK L., KUŻNA-GRYGIEL W., GONET B., PODRAZA W. 2010. Extremely low frequency magnetic field and the hatching rate of *Fasciola hepatica* eggs, the fecundity and survival of liver fluke-infected snail, *Lymnaea truncatula*. *Folia Biol. (Kraków)* **58**: 157-161. DOI:10.3409/fb58_3-4.157-161.
- KOŁODZIEJCZYK L., LASZCZYŃSKA M., MASIUK M., GRABOWSKA M., SKRZYDLEWSKA E. 2015. Immunoexpression of intermedia te filaments and morphological changes in the liver and bile duct of rats infected with *Fasciola hepatica*. *Biotech. Histochem.* **90**: 477-485. DOI: 10.3109/10520295.2015.1021712.
- KOMAZAKI S., TAKANO K. 2007. Induction of increase in intracellular calcium concentration of embryonic cells and acceleration of morphogenetic cell movements during amphibian gastrulation by a 50-Hz magnetic field. *J. Exp. Zool. Part A. Ecol. Genet. Physiol.* **307**: 156-162. DOI: 10.1002/jez.a.359.
- KUŻNA-GRYGIEL W., GONET B., JABOROWSKA M., KOŁODZIEJCZYK L. 2005. Effect of power network frequency magnetic field on embryonic development of *Ascaris suum* (Nematoda). *Folia Biol. (Kraków)* **53**: 101-105.
- LEE C.G., CHO S.H., LEE C.Y. 1995. Metacercarial production of *Lymnaea viridis* experimentally infected with *Fasciola hepatica*. *Vet. Parasitol.* **58**: 313-318. DOI: 10.1016/0304-4017(94)00725-R.
- TAYLOR E.L., MOZLEY A. 1948. A culture method for *Lymnaea truncatula*. *Nature*, **161**: 8.