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Morphometric analysis – effect of the radiofrequency interface of electromagnetic field on the size of hatched *Dermacentor reticulatus* larvae

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Abstract

Introduction. Electromagnetic radiation interactions with living systems have been one of the determining factors in biological evolution. This study investigates the effect of 900 MHz radiofrequency (RF) electromagnetics field (EMF) exposure of eggs on the development of Dermacentor reticulatus larvae.
Objective. The aim of the study was to determine whether the 900 MHz RF-EMF has the potential to influence the size of the body of the hatched larvae of D. reticulatus ticks.
Materials and method. Eggs from 3 fully engorged females of D. reticulatus were included in the test procedure. Altogether, four groups of eggs were designated, including eggs from each female. A RF-EMF frequency of 900 MHz was used. Eggs were exposed to EMF for different time periods (30, 60 and 90 minutes) in a dark, electromagnetically-shielded anechoic chamber. After irradiation, eggs were allowed to hatch in a climatic chamber. Randomly selected 200 larval individuals were measured to obtain basic morphological records. Four body traits, including total body length (TBL), length of gnathosoma with scutum (GSL), total body width (TBW), and width of the basis capituli (BCW) were measured.
Results. The D. reticulatus larvae hatched from eggs exposed for 60 minutes had demonstrably larger dimensions of all measured body traits, in comparison not only to the control unexposed group, but also to the other experimental groups.
Conclusions. The study shows particularly that artificial EMF used in smartphone technology impacts seriously on D. reticulatus larvae development.

Key words
electromagnetic field, development, tick, larva, body size, Dermacentor

INTRODUCTION

Electromagnetic radiation interactions with living systems have been one of the determining factors in biological evolution. Sunlight, lightning, cosmic rays, and the Earth´s magnetic field are all major sources of this natural electromagnetic field (EMF). During the past century, this environmental EMF has been sharply changed with the introduction of a vast and growing spectrum of man-made EMF-producing activities and products [1, 2], e.g. smart phones are important tools not only for communication, but also for bank transfers, newscasts, social media and numerous other conveniences. Concerns are being raised about the emission of radio frequency (RF) EMF from these devices and their broadcasting network as this market is still growing [3]. The frequency of RF-EMF is from 100 MHz to 6GHz, and the main sources of RF-EMF are wireless telecommunication base stations [4].

Numerous studies have shown the sensitivity of biological systems to external artificial RF-EMF. The radiation has an impact on surrounding flora as well as on vertebrate and invertebrate organisms [5]. RF-EMF affects the insects and arachnids [3, 6]. Insects play an important role in the balance of ecosystems. Balmori, [6] found that RF-EMF decreases the population size of insects and arachnids near the base stations. In human dwellings close to RF-EMF antennas, an absence of flies was found even in the summer period. Odemer and Odemer [7] discovered that mobile phone radiation (900 MHz RF-EMF) reduced the hatching ratio of the honeybee queen (*Apis mellifera*) up to 44%; however, mating success was not influenced. Several authors have described that extremely low EMF (50 Hz) significantly increased the wing size with the opposite directional asymmetry in *Drosophila subobscura* and *Drosophila melanogaster* [8, 9]. In addition,

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other studies affirm the RF-EMF have an impact on living systems [4, 10, 11, 12, 13, 14, 15].

Dermacentor reticulatus ticks poses a danger to humans and animals because it transmits the canine babesiosis agent (*Babesia canis*), several *Rickettsia* spp., the Omsk haemorrhagic fever virus, and Tick-borne encephalitis virus [16]. *D. reticulatus* is a hard tick species with a high reproduction rate. One fully engorged fertilized adult female can lay 3,000–7,000 eggs [16]. *D. reticulatus* female ticks provide the eggs with a secretion that prevents excessive water loss which causes the eggs to adhere in a cluster and probably protects them from fungal attack [17]. Only a few studies have shown the effect of RF-EMF on ticks. In *Hyalomma asiaticum*, low-power microwave modulated radiation significantly suppresses the development of both fed larvae and fed nymphs at 22 °C, whereas unfed specimens of all developmental phases were unresponsive [18]. Moreover, exposure delayed larval hatching from 3–20 days, increased the duration of activity of newly-moulted larvae by 17–24 days, and reduced the survival of unfed larvae and nymphs by 4–10 days [19]. Vargová et al., [20] observed the behavioural reaction of ticks due to 900 MHz RF-EMF exposure in which abnormal movements of ticks were displayed as sudden jerking movements. Vargová et al., [21] used the 900 MHz RF-EMF frequency on ticks that responded by moving towards the space irradiated by these waves (used in mobile phone communication) under laboratory conditions. While RF-EMF effects on behaviour in vertebrates and invertebrates have already been tested several times, no information on RF-EMF effects on body size or development of larvae of ticks exist.

In the current study, the RF-EMF frequency of 900 MHz based radiation was used to measure the size of selected body parts of hatched larvae from eggs after exposure to RF-EMF. The objective was to determine whether the 900 MHz RF-EMF has the potential to influence the size of the body of the hatched larvae of *D. reticulatus* ticks.

MATERIALS AND METHOD

Model organism. For testing, 3 fully-engorged adult female of *D. reticulatus* were used, collected by removing them from a dog in cooperation with the University of Veterinary Medicine and Pharmacy in Kosice. Engorged ticks were kept separately in polypropylene tubes in an environmental chamber at 20 °C and 90% relative humidity in a 16:8 light-dark regime. The oviposition started on day 23 after engorgement. Altogether, 3 clusters of eggs were obtained from 3 females.

Experimental setup. Egg clusters from each female were divided into 4 clutches with an average of 500 eggs each. The egg clutches were sorted into 4 groups, with eggs from all 3 females in each group (Fig. 1). The eggs were placed on Petri dishes with moistened filter paper to ensure optimal moisture and thus protect eggs from drying out. All experiments were performed in an anechoic chamber [see 20]. Petri dishes with eggs were placed into an anechoic chamber and exposed to RF-EMF. Three groups were exposed to the 900 MHz EMF frequency with different exposure times. The first group of eggs was exposed for 30 minutes, the second group for 60 min., and the third group for 90 min. The fourth group of eggs was the control group that was also placed in an anechoic chamber, but without exposure of RF-EMF. At the end of the experiments, the clusters were placed in polypropylene tubes with moistened filter paper and placed in an environmental chamber at 20 °C and 90% relative humidity in a 16:8 light-dark regime. Larvae began to hatch 15 days after oviposition (Fig. 2). Hatched larvae were stored in 70% ethanol.

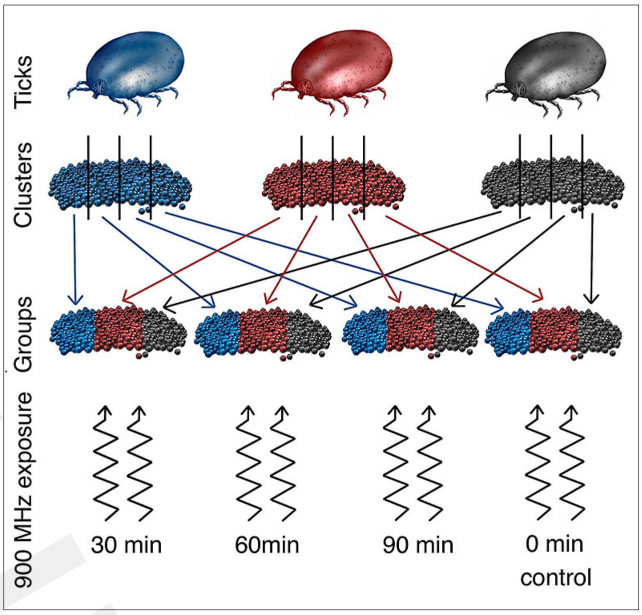


Figure 1. Schematic design of experimental groups of eggs laid by *Dermacentor reticulatus* ticks. Four groups of eggs were combined from clusters of eggs laid by 3 females. Three experimental groups of eggs were exposed to a 900 MHz electromagnetic field; a group of unexposed eggs was included as the control group.

Figure 2. Eggs of *Dermacentor reticulatus* ticks with developed larvae inside. Photo was taken on the day of hatching. Scale bar showing 100 µm.

Electromagnetic conditions. By using an analogue signal generator (model N5183A, Agilent, MY) the EMF radiation of frequency 900 MHz was generated. The object under test was irradiated by a Double-Ridged Waveguide Horn Antenna (HF907, Rohde and Schwarz, Munich, DE) with vertical polarization of the electric part of the RF field. Inspired by Salzburg Resolution on Mobile Telecommunication Base Stations [22], the power density of 1 mV/m^2 was applied at the location of the object under the test. The setup calibration was performed,using a spectrum analyser [Spectran HF-60105, Aaronia, DE].

Thermal conditions. The temperature of the egg groups during the experiment was stable, between 15.5–16.5 °C, and monitored by an infrared camera (FLIR T335, Flir Systems AB, Sweden). The room temperature and humidity were monitored by a Klimalogg Base multimeter. The temperature in the anechoic chamber was 20 °C, humidity – 45%.

Morphometric analysis. Fifty larvae were collected from each experimental group. A digital photo of the individual larva was taken from ventral side under a microscope using the Optic Vision Lite 2.1 optical system. A total of 4 body dimensions were measured with 0.01mm accuracy. The total body length (TBL), length of *gnathosoma* with sclerotized dorsal plate (*scutum*) (GSL), total body width (TBW), and width of the *basis capituli* (basal portion of the capitulum on which the mouthparts are attached) (BCW) was measured. TBL was measured from the apical part of the hypostome to the caudal part of festoons, and GSL was measured from the apical part of the hypostome to the caudal part of *scutum*. Perpendicular to this measure was TBW, which was measured at the widest part of body (Fig. 3).

Statistical analysis. A descriptive statistics table was constructed using the dataset of measures of morphometry dimensions. The consistency of the collected experimental measures and evidence of the larvae development in different time doses time was presented by means of the data distribution kernels in the violin plots. To discover whether the irradiation in different time groups were significantly different or similar, the Tukey's test was used. To visualize the high dimensional morphometry of larvae development, 2 dimensionality reduction techniques were used: the principal component analysis (PCA) and t-distributed stochastic neighbour embedding (t-SNE) space. The significance of the irradiation is interpreted in the form of the clusters in reduced data-space.

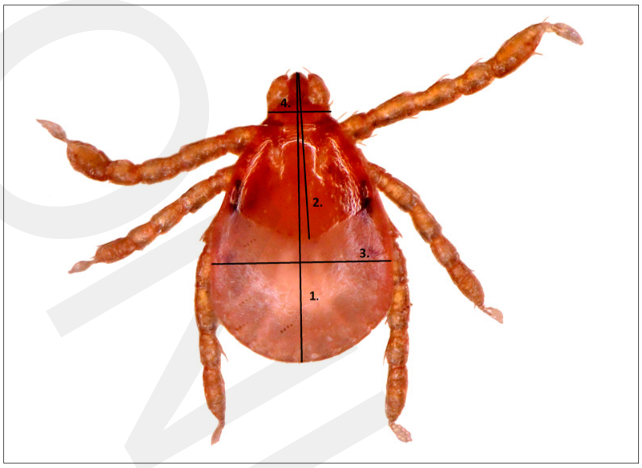


Figure 3. Larva of *Dermacentor reticulatus,* x500 magnification. Body trait dimensions measured in this study are indicated by black lines as follows: 1) total body length (TBL); 2) length of *gnathosoma* with *scutum* (GSL); 3) total body width (TBW); 4) the width of *basis capituli* (BCW). Trait No. 2 is diverted in the picture for illustration, otherwise it overlaps with trait No. 1.

RESULTS

The experimental sample consisted of the morphometry dimensions measured on 200 hatched larvae originating from 4 groups. Altogether, 800 dimensions were measured, 4 parameters on each individual tick. Besides the control set (N=50), they were irradiated for 30 (N=50), 60 (N=50) and 90 (N=50) minutes, respectively. Measurements of the irradiated egg clutches were compared with the control set measures. Descriptive statistics of larvae development morphometry measurements are shown in Table 1, Figure 4, and Graph 1.

The most significant differences between the measured parameters occurred in the 900 MHz group exposed for 60 min. The ticks *D. reticulatus* exposed for 60 minutes had demonstrably larger body dimensions of all measured parts,

Table 1. Descriptive statistics of larvae development morphometry measures. TBL – total body length, GSL – length of *gnathosoma* with *scutum*, TBW – total body width, BCW – width of *basis capituli*

Body dimension	Dose time (s)	N	Mean (mm)	Standard Deviation (mm)	Lower 95% CI of Mean (mm)	Upper 95% CI of Mean (mm)	Minimum (mm)	Median (mm)	Maximum (mm)
TBL	0	50	0.693	0.012	0.690	0.697	0.669	0.696	0.724
TBW	0	50	0.367	0.033	0.358	0.376	0.158	0.373	0.398
BCW	0	50	0.153	0.033	0.144	0.162	0.128	0.150	0.375
GSL	0	50	0.399	0.010	0.396	0.402	0.377	0.398	0.424
TBL	30	50	0.678	0.012	0.675	0.682	0.653	0.678	0.711
TBW	30	50	0.362	0.015	0.358	0.366	0.335	0.363	0.389
BCW	30	50	0.139	0.007	0.137	0.140	0.126	0.138	0.162
GSL	30	50	0.387	0.009	0.384	0.389	0.367	0.385	0.403
TBL	60	50	0.715	0.014	0.711	0.719	0.679	0.715	0.748
TBW	60	50	0.378	0.014	0.374	0.382	0.348	0.377	0.409
BCW	60	50	0.145	0.007	0.143	0.147	0.132	0.144	0.163
GSL	60	50	0.406	0.014	0.402	0.410	0.379	0.407	0.455
TBL	90	50	0.636	0.017	0.631	0.641	0.601	0.635	0.672
TBW	90	50	0.344	0.016	0.339	0.348	0.300	0.343	0.380
BCW	90	50	0.135	0.009	0.133	0.138	0.115	0.136	0.156
GSL	90	50	0.363	0.014	0.359	0.367	0.333	0.364	0.397

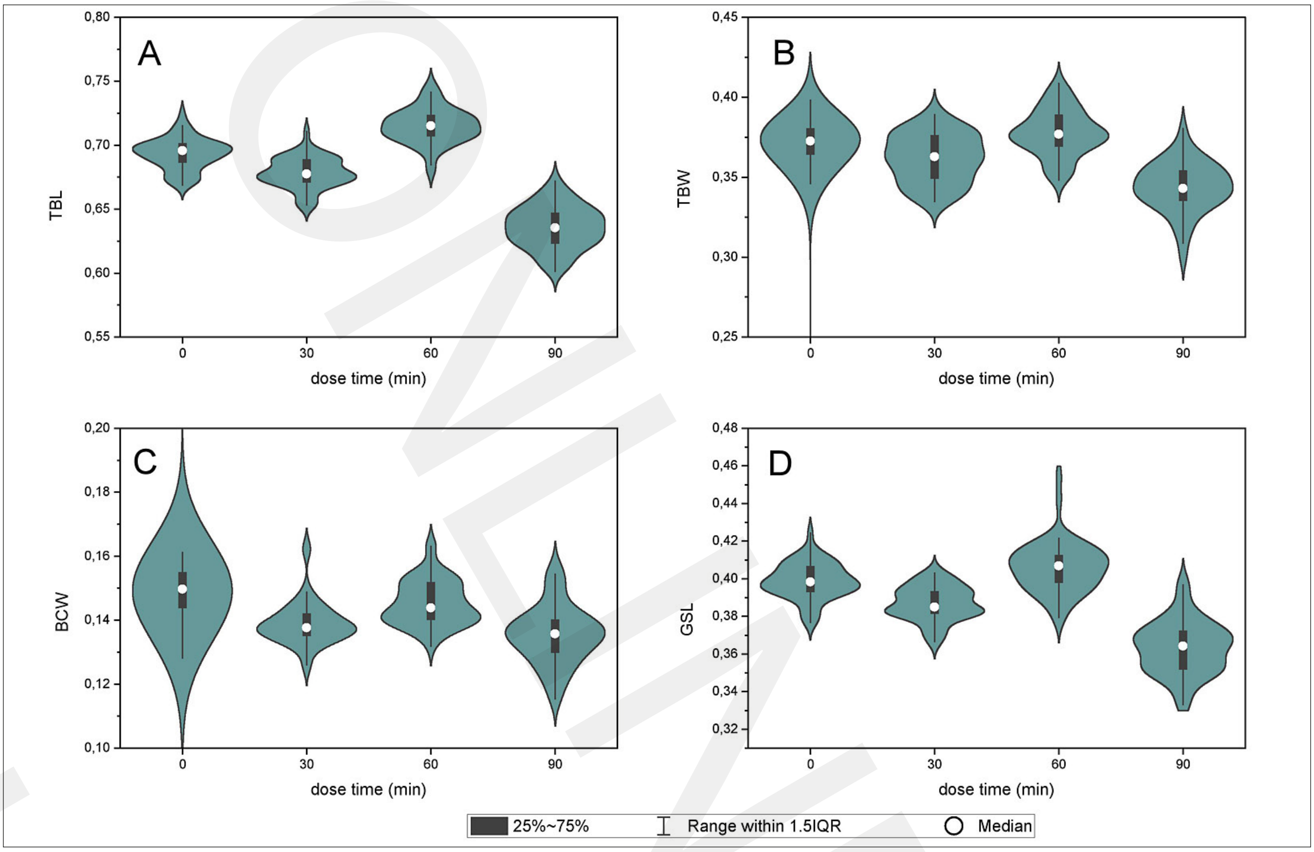
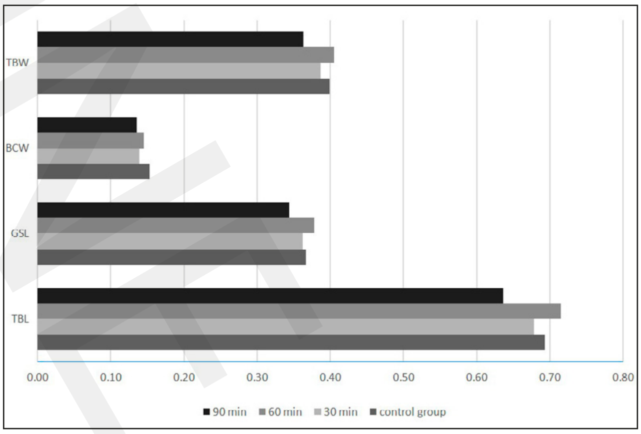


Figure 4. Quartiles and data distribution kernels describing the measured body trait dimensions of *Dermacentor reticulatus* larvae hatched from eggs exposed to a 900MHz electromagnetic field with different exposure time.
TBL – total body length; GSL – length of *gnathosoma* with *scutum*; TBW – total body width; BCW – width of *basis capituli*



Graph 1. Comparison of mean values of measured body traits in all experimental groups.
TBL – total body length; GSL – length of *gnathosoma* with *scutum;* TBW – total body width; BCW – width of *basis capituli*).

not only as a control group, but also in the other groups. The greatest difference was seen in the length of the gnathosoma and scutum where the average was 0.72 mm, compared to the larval control group, where this parameter was 0.69 mm. Length of scutum in the 60 min exposed group, on average, was 0.01 mm larger than in the control group. Equally, larger dimensions were recorded in the width of the scutum – 0.02 mm. There was no significant difference in the width of the basis capituli, No deformations or other anomalies were observed.

The significance of the irradiation impact on the larvae development was validated by the ANOVA Tukey Test at significance level 0.05. The probability and significance are shown in in Table 2.

To show the different variability of measured parameters, principal component analysis (PCA) was conducted. Four principal components (PC1-C4) cover 100% variability of measured values. The proportion of variance of the components, their eigenvalues and feature contributions are shown in Table 3, and informative projection of the most important components PC1 vs PC2 is shown in Figure 5. Colours are used to show the contributions of irradiation lasting to the component values. There are 4 main areas on the graph that belong to the dose time, which indicates that there is clear evidence of irradiation of the egg clutches development.

DISCUSSION

D. reticulatus is a hard tick with unique biology and ecology; it is extremely tolerant to a wide spectrum of unfavourable conditions and has a high adaptivity, which led to its spreading into new areas. *D. reticulatus* ticks play an important role in the transmission of pathogens that can potentially be transmitted by several viruses, bacteria and protists. *D. reticulatus* females lay up to 7,200 eggs and the adults are very resistant to the external environment [16]. Although great progress has been made in knowledge of the eco-epidemiology of this species, several gaps still need to

Table 2. Test of significance of irradiation impact on larvae development

Dependent variable	Factor levels	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
TBL	30_0	-0.01502	0.00274	7.73821	≈0	0.05	1	-0.02213	-0.00791
	60_0	0.02181	0.00274	11.23431	0	0.05	1	0.01469	0.02892
	60_30	0.03683	0.00274	18.97252	≈0	0.05	1	0.02971	0.04394
	90_0	-0.05747	0.00274	29.61084	≈0	0.05	1	-0.06459	-0.05036
	90_30	-0.04245	0.00274	21.87263	0	0.05	1	-0.04957	-0.03534
	90_60	-0.07928	0.00274	40.84515	0	0.05	1	-0.08639	-0.07217

Dependent variable	Factor levels	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
TBW	30_0	-0.00519	0.00419	1.74952	0.60405	0.05	0	-0.01605	0.00568
	60_0	0.01072	0.00419	3.61603	0.0546	0.05	0	-1.44E-04	0.02158
	60_30	0.0159	0.00419	5.36555	0.00112	0.05	1	0.00504	0.02676
	90_0	-0.02318	0.00419	7.82181	≈0	0.05	1	-0.03404	-0.01232
	90_30	-0.018	0.00419	6.07229	≈0	0.05	1	-0.02886	-0.00714
	90_60	-0.0339	0.00419	11.43784	0	0.05	1	-0.04476	-0.02304

Dependent variable	Factor levels	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
BCW	30_0	-0.01413	0.00356	5.61807	»0	0.05	1	-0.02335	-0.00491
	60_0	-0.00773	0.00356	3.0718	0.13469	0.05	0	-0.01694	0.00149
	60_30	0.0064	0.00356	2.54627	0.27621	0.05	0	-0.00281	0.01562
	90_0	-0.01773	0.00356	7.0515	»0	0.05	1	-0.02695	-0.00852
	90_30	-0.00361	0.00356	1.43343	0.74168	0.05	0	-0.01282	0.00561
	90_60	-0.01001	0.00356	3.9797	0.02742	0.05	1	-0.01923	-7.93E-04

Dependent variable	Factor levels	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
GSL	30_0	-0.0119	0.00236	7.12985	≈0	0.05	1	-0.01802	-0.00579
	60_0	0.00733	0.00236	4.39142	0.01163	0.05	1	0.00121	0.01345
	60_30	0.01923	0.00236	11.52127	0	0.05	1	0.01312	0.02535
	90_0	-0.0354	0.00236	21.20412	0	0.05	1	-0.04152	-0.02928
	90_30	-0.0235	0.00236	14.07427	0	0.05	1	-0.02961	-0.01738
	90_60	-0.04273	0.00236	25.59554	0	0.05	1	-0.04885	-0.03661

MeanDiff - mean difference, SEM - standard error of the mean, Prob – probability, Alpha - significance level, Sig – significance, LCL – lower bound of confidence, UCL – upper bound of confidence, Sig equals 1 indicates that difference of means is significant at level 0.05, Sig equals 0 indicates that difference of means is not significant at level 0.05. TBL – total body length, GSL – length of *gnathosoma* with *scutum*, TBW – total body width, BCW – width of *basis capituli*

Table 3. Principal component analysis of experimental data. TBL – total body length, GSL – length of *gnathosoma* with *scutum*, TBW – total body width, BCW-width of *basis capituli*

Principal Component Number	Proportion of variance	Eigen-values	TBL	TBW	BCW	GSL
PC1	57.35%	2.29405	0.62309	0.45064	0.14546	0.62251
PC2	32.36%	1.29448	0.10057	-0.55556	0.81796	0.11038
PC3	6.89%	0.27565	-0.32259	0.69877	0.5565	-0.31299
PC4	3.40%	0.13582	0.7054	0.00070	0.00939	-0.70875

be filled. Several important biotic and abiotic factors have already been identified as affecting the biology of this tick species. In several publications, an electro-magnetic field was found to affect the behavioural features of *Dermacentor* ticks, and also showed different affinity to the EMF frequency [21]. The occurrence of man-made electromagnetic fields is constantly increasing in the environment, not only in urban but also in the natural habitat. In recent years, the public has been widely and increasingly using mobile phones with 900

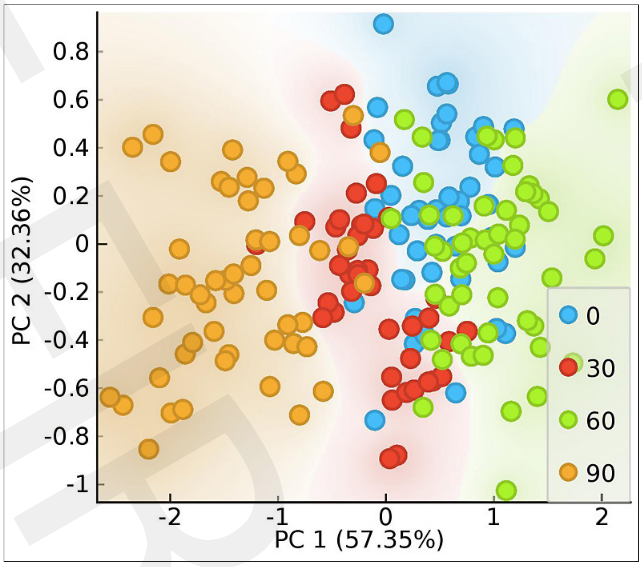


Figure 5. Projection of principal component analysis. PC1 vs PC2 components are shown

MHz frequency [6]. The effect of this frequency on biological objects is still insufficiently investigated. The current study aimed investigate the of effect of the 900 MHz frequency on the development of larvae in eggs, and investigation the morphological differences in the hatched larvae. This study is the first scientific report on the effect of RF- EMF on the body size of *D. reticulatus* larvae. The studied 900 MHz RF-EMF frequency is the one typically emitted by telecommunication antennas, mobile phones, and other transmitters, which are very abundant in the environment.

Morphometric analysis data clearly indicated that radiation has a significant impact on the evaluated body traits of *D. reticulatus* larvae. Surprisingly, the morphometry results obtained from EMF exposed larvae were not consistent. It cannot concluded whether the effect of EMF on body trait enhances or limits the growth of the larvae, compared to the non-exposed control. In the eggs exposed for 30 and 90 minutes exposure, a limitation in growth was observed in all parameters, and 60 minutes exposure had a significant growth-enhancing effect in all observed parameters. The impact of EMF was also studied by Korotkov et al. [18] who showed that the hatching and metamorphosis of the *Hyalomma asiaticum* tick is affected by low-power (20 microW/cm^2) microwave-modulated radiation.

Morphometric analysis without the influence of RF-EMF was performed on *D. reticulatus* ticks' larvae. The size and width of larval tarsus were compared between the tick populations of Poland and Slovakia, with significant differences found between populations [23]. Studies dealing with larval development were focused mainly of the presence of body malformations. Environmental stressors, such as high temperature, humidity, use of chemicals in the environment, and host resistance to tick infestation, are among the main causes of morphological abnormalities in ticks [23, 24, 25]. The effect of high relative humidity on larval development showed harmful influence on the embryonic development of *Hyalomma marginatum* [26]. Experiments by Buczek et al. [23] confirmed that colchicine, a cytotoxic alkaloid of plant origin, caused the death of eggs and embryos, disruption of larval hatching, and the development of morphological anomalies in larvae. Morphological abnormalities (heteromorphosis of leg segments, oligomely, heterosymely, atrophy, altered shape, etc.) were found in 0.2% of the larvae. Developmental abnormalities were also observed in the environment with high anthropogenic pressure, without identification of exact cause of the malformations [27, 28]. Morphological anomalies can occur in any developmental stage (i.e. larva, nymph or adult) in natural populations of ixodid ticks. Malformations were more often expressed in the *Ixodes persulcatus* than *Dermacentor* and *Haemaphysalis* ticks studied in the Altai Republic in Russia. When considering the presence of tick-bone encephalitis virus in ticks, the percentage of infected abnormal ticks was lower than of *Ixodes* without exoskeleton anomalies [29].

Mirabolghasemi and Azarnia described the biological effect of EMF on the developmental stages of *Drosophila melanogaster* eggs, and the first, second and third instar of larvae. Larvae exposed to EMF showed a higher incidence of abnormalities and was influenced by the duration of exposure. Their findings indicate a significant increase in the number of abnormal adult flies, whereas no significant increase was observed in the group arising from eggs exposed to EMF [9]. Panagopoulos et al. [30] described the reproductive capacity of *Drosophila melanogaster*, and discovered that the RF-EMF 900 MHz frequency decreased reproduction of the insect by 50%–60%.

Current understanding of the life-cycle traits and ecological aspects of *D. reticulatus* is rather limited compared to the well-studied *Ixodes* spp. ticks. This is mainly due to the lack of information concerning the developmental stages which are largely hidden, and it is almost impossible to collect them in the field. Morphological studies of the body traits of larvae are limited to laboratory-reared ticks.

CONCLUSION

The presented study reports for the first time the morphological changes in *D. reticulatus* larvae hatched from EMF exposed eggs. Further studies with longer exposures, other frequencies or intensities of EMF, repeated exposures or exposure of other developmental stages also focusing on developmental malformations, would have a high scientific value.

Acknowledgement

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