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Study on the dynamics of Warble Fly Infestation of the Red Deer and Roe Deer Herds in Szigetköz, Ravazd, and the area of Tarján (2005-2020)

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1. AIMS

The objective of our research was to assess the warble fly infestation of red deer and roe deer herds in the study site (Szigetköz, as well as the area of Tarján and Ravazd) between 2005 and 2020 in order to collect data about the prevalence of these parasite species in less studied areas of Hungary, and their impact on red deer and roe deer herds, as well as to reveal links that can be utilized in the practice of game management (potential protection options, wildlife management). Regarding the above, the specific objectives of our study were as follows:

- Assessment of *Hypoderma diana* and *Hypoderma actaeon* infection dynamics in the red deer and roe deer herds in Szigetköz (2005-2020).
- Assessment of *Hypoderma diana* and *Hypoderma actaeon* infection dynamics in the red deer and roe deer herds in Tarján and its surroundings (2011-2020).
- Assessment of *Hypoderma diana* and *Hypoderma actaeon* infection dynamics in the red deer and roe deer herds in Ravazd and its surroundings (2014-2020).
- Testing the histologic method developed for *Gasterophilus* botfly species for the identification of certain micromorphological characteristics of *Hypoderma diana* and *Hypoderma actaeon* larvae.
- Testing the usability of Quantitative Parasitology 3.0 (QPWeb) statistical analysis program for the determination of hypodermosis.

2. MATERIALS AND METHODS

2.1. Test sites

Samples were collected in hunting areas of Győr-Moson-Sopron and Komárom-Esztergom counties between 2005 and 2020. Samples of Győr-Moson-Sopron county were obtained from the hunting areas of Győr and Ravazd Forestry of Kisalföldi Forestry Closed Company. and that of the Felső-Szigetközi Hunting Association. In some occasions samples from the Szigetköz Hunting Association could also be processed. The Alsó-Szigetköz hunting area of Kisalföldi Forestry Closed Company (hunting area code of No. 100350) was located in Szigetköz, between Dunaremete and Vámosszabadi, in the forebank of Danube, and protected side next to the Danube dam, as well as in the municipality borders of Ravazd, Pannonhalma, Sokorópátka and Tényő.

Komárom-Esztergom county samples were collected in the big game hunt area in Dunazug, in the surroundings of Tarján, primarily in the areas of Management of Gerecse Forestry of Vérteserdő Closed Company.

The present study has already been proceeded by the assessment carried out in the hunting season of 2005/2006. That time the study just only covered the red deer and roe deer herds of so-called Szigetköz region. In the hunting season of 2011/2012 the study sites were extended also to game management area of Dunazug since earlier no such assessment was carried out around the municipality of Tarján. Our recent research, the site of which expanded with the area of Ravazd Forestry as well, was carried out from the beginning of September, 2014 to the end of February, 2020, during the hunting seasons of 2014/2015, 2015/2016, 2016/2017, 1017/2018, 2018/2019 and 2019/2020.

2.2. Sample collection and preservation methods

Samples were mainly collected from animals shot in hunting season however outside the hunting season we also had opportunities to examine animals died in road accidents or ones shot due to different wildlife health aspects.

By palpating we located the larvae then the skin were incised with a scalpel (Aesculap BA20), and the larvae were removed with anatomical forceps. The removed skins of the locally sold specimens were also examined and the structures under the skin were also examined and all *Hypoderma*-larvae found there were collected. Professional hunters of the local game management units and the staff working there also facilitated the collection of larvae.

Recorded data included the site and time of the study, animal species, gender, age, ear tag code, site and time of the kill, gutted weight, condition and the number warble fly larvae as well as the nature of necropsy symptoms, and other observations. Several cases data necessary for the identification of specimen not or just incompletely were available which made it difficult to evaluate our studies. In roe deer, there were not too much opportunity to examine the bucks since the period of time when larvae stay under the skin is mostly outside the buck hunting season.

For each host animals the larvae were placed in separate screw-top jars filled with 20% ethyl alcohol and stored in a refrigerator. Although this

method differs significantly from the ones described in the scientific literature according to which the larvae should be conserved in 80% ethyl alcohol solution (Fehér, 1975). However considering the large number of samples it was not seemed to be a feasible way. (As earlier we found the 20% ethyl alcohol could conserve the larvae without any problems. This finding was supported by the fact that samples collected even in the 2005/2006 season remained in good condition. Storing the samples refrigerated and processing as soon as possible prevented larval maceration therefore based on their morphology characteristics they could be identified without exception.

2.3. Methods used for taxonomic identification and histology examination of the larvae

The taxonomic identification of the larvae was performed at the Department of Animal Sciences (earlier Animal Physiology and Animal Health), Széchenyi István University (Mosonmagyaróvár), using a WILD-LEITZ-LEICA M420 stereo microscope. The larvae were identified by using the determinant keys published by Minář (2000), Papp and Szappanos (1992), according to the following morphological principles: the corona above the pseudoencephalon is formed by at least two rows of rarely absent, small spines. Spines on the dorsal side are much smaller than on the ventral side. The peritremas are flat or tunnel-shaped. The histology method developed by Egri (1987) and Egri Bné et al. (1992) for examining of horse botfly (*Gasterophilus intestinalis*) larvae and also the recent histology results published by Egri et al. (2019) were appropriated in the histological examinations of larvae.

For the later purpose 2472 third instar (L3) warble fly (*Hypoderma diana* and *Hypoderma actaeon*) larvae were collected from 52 red deer and 31 roe deer carcasses obtained in Szigetköz (North West Hungary) in the hunting season of 2018/2019. Taxonomic identification of the larvae was performed as described earlier.

Larvae were conserved in a 10% formaldehyde solution for 10 days. Then the histology method described by Egri, B (1987) and Egri Bné et al. (1992) for histologic examination of horse bot fly larvae was applied. Larvae were cut longitudinally. The second fixation was performed in 10% formaldehyde solution (for 16 hours). Then larvae were washed for 30 minutes. For dehydration a solution of 96% propyl alcohol (1 hour), acetone with anhydrous copper(II)-sulphate (4 x 1 hour) and finally xylene (3 x 1 hour) was used. Then the larvae were embedded in beeswax (ratio 1:12.5). Hematoxylin and eosin dye was used for staining. A combination of Nikon Eclipse Ni-U light microscope and a Nikon DS-U3 digital camera control unit was appropriated in the histology.

2.4. Data processing

For the analysis of our results the Quantitative Parasitology 2.0 (QP 2.0) package (Rózsa, et al. 2000; Reiczigel and Rózsa, 2001) was used in the 2005/2006 hunting season then from the 2010/2011 season we changed it to a newer version Quantitative Parasitology 3.0 (QP 3.0) package Reiczigel and Rózsa, 2005).

We calculated the prevalence, the mean intensity and the confidence interval. The level of aggregation was expressed in numbers by using discrepancy index. Chi-square test and Fisher exact test were applied for comparing prevalence rates. For comparing the median intensity of two samples the bootstrap test, while for the comparison of median intensity of two or more samples the Mood's median test was used.

3. SCIENTIFIC RESULTS

- 1. In the study period of 2005-2020 the prevalence of hypodermosis found between 68,6% and 95,4% among the red deer in Szigetköz, in 2011-2020 it was 70-83,80% in Tarján and its surroundings, while in 2014-2020 we measured 78,9-90% in Ravazd and its surroundings. In the above mentioned study periods we found 70-89,6% in Szigetköz, 72-84,4% in Tarján and its surroundings, and 70,6-91,9% in Ravazd and its surroundings.
- 2. By using quantitative parasitology methods (QP 2.0, then from the hunting year of 2011/2012 QP 3.0) we compared the data obtained from red deer and roe deer in different years and areas. Highest infection rates were found in red deer and roe deer from the Szigetköz, however these values did not differ significantly from values measured in the other two study sites which showed about the same values.
- 3. We used firstly the histological method of Egri (1987) and Egri Bné et al. (1992) approved to screening horse bot fly (*Gasterophilus intestinalis*) larvae for the light microscopic examination of warble fly larvae. We confirmed that the special histological method used for observation of horse bot fly larvae could also be appropriated for warble fly larvae, thus we found potential options to map the fine internal structure of warble fly larvae. To have more knowledge concerning these structures may help us to gain in deer insight to the larval metabolism and development. Our investigation revealed that the general appearance and internal structures of *Hypoderma diana* and *Hypoderma actaeon* larvae are quite similar.

4. LIST OF PUBLICATIONS BY THE AUTHOR ON THE TOPIC

Husvéth, B., Egri B. (2021): Retrospective Study on the Occurrence of Warble Fly Infestation (Hypodermosis) of the Red Deer and Roe Deer Herds in North-North-West Hungary (Szigetköz, District of Ravazd and Tarján). INTERNATIONAL JOURNAL OF ZOOLOGY AND ANIMAL BIOLOGY 4(2): p. 000290. [IF.: 1.7594]

Egri, B., Bieber M., Husvéth, B. (2019): Notes on Micromorphology of Third Instar Larvae of *Hypoderma actaeon* (Brauer, 1858) and H. diana (Brauer, 1858) (Oestridae, Hypodermatidae). ACTA MICROSCOPICA 28(5): 933-937. [IF.: O.16]

Husvéth, B., Egri, B. (2014): A bőrbagócsosság elterjedése Tarján és környéke gímszarvas- és őzállományaiban VADBIOLÓGIA 16: 63-67.

Egri, B., Husvéth, B. (2007): Először a Szigetköz őz- és gímszarvasállományának bőrbagócs-fertőzöttségéről [The first time about the warble fly infestation of roe-deer and red deer in Szigetkoz] MAGYAR ÁLLATORVOSOK LAPJA. 129(2): 21-127. [IF: O.196.]

Congress summary

Egri, B. and Husvéth, B. (2006): On the infestation of warble fly-larvae in the Antlered Ruminants in Szigetköz Region. In: Suwa, Masaaki (szerk.): 6th International Congress of Dipterology. 23-28 September, 2006. Fukuoka, Japan. p. 68.