THESIS OF THE Ph.D. DISSERTATION

EPIDEMIOLOGICAL SITUATION OF NASAL BOTFLY INFESTATION IN THE HUNGARIAN ROE DEER POPULATION AND BIOLOGICAL CHARACTERISTICS OF THE PARASITE

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MOSONMAGYARÓVÁR
2009

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1. Objectives

The objective of my research was the evaluation of botfly infestation in the Hungarian roe deer population in order to collect valuable data regarding the distribution of the parasite and its effect on the roe deer population, and to reveal any relationships that could be helpful in wildlife management.

During my research I have searched the answer for the following questions:

1. The larvae of which botfly species can be found in the Hungarian roe deer population?
2. Can the distribution of botfly species considered general in the Hungarian roe deer population?
3. What are the values of main indicators of infestation (prevalence, average intensity and median intensity)?
4. What are the annual dynamics of the botfly infestation in the roe deer population?
5. Are there any differences in the indicators according to age and gender?
6. Are there any differences in the infestation indicators in differently aged male roe deer?
7. When do the different stages of the larval development occur in the Hungarian roe deer population?
8. How do the monthly dynamics of the prevalence, average intensity and median intensity change?
9. Do the infestation indicators of various ecotypes (forested areas, fields) of the host populations differ (are these populations of different quality from the wildlife management point of view), are there any differences in their level of infestation?
10. Does the level of infestation have any effect on the host organism (bodyweight, trophy weight)?

2. Materials and Methods

One of the aims of the study was to obtain representative samples regarding the level of infestation of roe deer populations of various qualities, such as the ones living in forests and open grasslands. For this, during the survey I have designated the territories and counties of sample origin according to the indices of trophy evaluation (especially prize trophy ratio). When choosing the territories and counties, I have taken into consideration the existence of the sylvan and grassland ecotypes. Based on these criteria, I have collected samples from 10 counties (Baranya, Bács-Kiskun, Békés, Fejér, Komárom-Esztergom, Somogy, Jász-Nagykun-Szolnok, Tolna, Veszprém, and Zala counties) with the help of professional hunters and other specialists. The investigations were performed from April 2002 to April 2005, hence during the hunting seasons 2002/2003, 2003/2004, and 2004/2005.

During the survey 647 males, 211 females and 100 fawns, therefore a total of **958 individuals** have been examined.

In case of male animals I have recorded the time (month) and place of the kill, along with the age of the animal, while in case of females and fawns the place of the kill and the age of the animal. In case of most males the official weight trophy and the eviscerated body weight have also been recorded. These values were recorded in order to investigate the effects of the various levels of infestation on individual productions (trophy weight, body weight). In the case of bucks, the so-called "small skulls" were examined (the horizontal section from the dorsal angle of the protuberantia occipitalis externa to the cranial end of the nasal bone).

The larvae found in each animal were stored in a vessel with all the data regarding the given individual.

In case of female animals and fawns, in order to examine the nasal and pharyngeal cavities, the cranium was split in two along the sagittal sulcus.

The identification of the species and larval stadium was performed using a stereo microscope in case of stage two and three larvae, and a stereo and a laboratory microscope in case of stage one larvae.

3. Results and Discussion

3.1. Botfly species found in the Hungarian roe deer population

Regarding the botfly species occurring in roe deer the results of my investigations were similar to those of other national and international survey, as in the examined individuals I could only identify *Cephenemyia stimulator* (CLARK, 1815) larvae.

SUGÁR (1974, 1975, 1978a, 1978b) reported the occurrence of *Pharingomyia picta* (MEIGEN, 1824) in roe deer. On the other hand, he managed to demonstrate the presence of only two larvae in a single animal, which could be only an accidental infestation, similarly to the occurrence of *C. ulrichii* in roe deer from Finland (NILSSEN *et al.* 2008).

The FAHRENHOLZ rule could be another argument of the accidental infestation, as the two botfly species belong to the same genus, just like the red deer and roe deer: the red deer is a member of the old world deer subfamily *(Cervinae)*, while the roe deer belongs to the new world deer subfamily *(Odocoileinae)* (PETZSCH, 1973; DOUZERY & RANDI, 1997; PITRA *et al.*, 2004). The co-evolution theory of host and parasite species (RÓZSA, 1989, 2005a) also implies that *Ph. picta* is not a normal parasite of roe deer.

3.2. Incidence of botfly infestation, and the level of infestation present in Hungary

During my investigations I found evidence of botfly infestation in all of the ten counties included in the survey. The territories of sample origin were representative for the Hungarian populations, for the sylvan and grassland ecotypes alike, therefore it can be concluded that *C. stimulator* can be considered a generally prevalent parasite in the Hungarian roe deer population.

On the other hand, I have found differences in the level of infestation among the investigated counties, as well as between the results of investigations performed on Hungarian and other European roe deer populations.

Based on the overall results the extensity of infestation of the Hungarian roe deer population was 34.6%, which significantly differ from the previous findings (66.7%) of SUGÁR (1975), but corresponds with the earlier findings of KIRÁLY & EGRI (2002, 2003): 34.8 and 35.2%, respectively. Investigations performed on French roe deer populations provided very similar findings (MAES & BOULARD, 2000) regarding the level of prevalence. The prevalence values were slightly higher in case of German populations, as reported by BARTH *et al.* (1976). Three different surveys on Czech roe deer populations provided significantly different values (LAMKA *et al.*, 1997; VACA, 2000; CURLIK *et al.,* 2002).

The average intensity of larval infestation was 8.87 larvae/host. This value is not very different from the findings of other Hungarian reports (KIRÁLY & EGRI 2003, 2004). Even tough SUGÁR (1975, 1978b) reported higher levels of infestation of the Hungarian roe deer populations, only a very limited number of samples (n=24) were analyzed. The average intensity of larval infestation did not differ significantly from the values reported by other foreign surveys (BARTH *et al.*, 1976; VACA, 2000; CURLIK *et al.*, 2000).

The level of median intensity observed during my study was 5 larvae/host. This result could not be compared to the findings of other investigations, because this index was not used in previous publications.

As expected, the value of the infestation indices was not homogenous among the investigated territories. The following tendency could be observed: the three infestation indices decreased with the increase of the proportion of forested areas, and it increased along with the increase of population density. However, these differences were not significant.

3.3. The yearly dynamics of the main indices of botfly infestation

The performed bio-mathematical analysis did not reveal significant differences regarding any of the infestation indices, with the prevalence of P=0.693, average intensity P=0.657 (2002-2003), P=0,586, (2002-2004), P=0,451 (2003-2004), while the median intensity was P=0.195. Based on these values it can be concluded that the main indices do not change on a yearly basis, at least the ones concerning the infestation characteristics of the roe deer population of Tolna County.

Currently there are no Hungarian or foreign reports regarding the annual dynamics of the botfly infestation. Investigations regarding the antiparasitic medication have only confirmed the results of my survey: there were no significant changes in the annual infestation indices in successive years, and that following the interruption of medication the prevalence of the infestation increased to a value very similar to the one before the medication (LAMKA et al., 1997; HAUGERUD et al., 1993).

3.4. Comparison of the infestation indices of differently aged male groups

There are several foreign reports regarding the differences of the infestation indices of differently aged male groups of various wild and economic species. Based on these reports it was expected that in case of roe deer the differences in the infestation indices in different aged groups would be more evident. I found that the explanation of this phenomenon lies in the territorial behavior of deer. According to BOBEK (1977), the size of the territory is closely correlated with the age of the deer and the food resources, therefore the older and stronger individuals obtain the best territories, while the young ones are very frequently forced to migrate. CSÁNYI *et al*. (2003) obtained similar results when analyzing the Hungarian populations.

Even tough the average and median intensity values showed a decreasing tendency as the animals got older, in contrast to the hypothesis mentioned earlier, I did not find significant differences among the infestation indices of the young, middle aged and old male animals.

During their investigations of the Czechoslovakian roe deer population DYK & DYKOVÁ (1962) obtained similar findings, but without significant tendencies: according to their findings, the infestation indices of the older individuals were lower. VACA (2000) also concluded that the prevalence and average intensity of the larval infestation in the Czech populations is higher in case of one-year old animals, than in the older groups.

In contrast to the above mentioned findings, according to other Hungarian researchers (SUGÁR, 1978a) there are no differences among the infestation indices of differently aged animal groups.

3.5. Infestation indices of the two sexes

During my analysis I did not find demonstrable differences between the infestation indices of the two sexes. The overall prevalence for females was 33.60%, while in case of the male animals it was 34.60%. This finding could be observed in case of the median intensity as well (5 larvae/host in case of both sexes). Only the value of the average larval intensity was significantly higher in case of male animals, but this finding cannot be interpreted as a demonstrable difference in the infestation level of the two sexes (RÓZSA, 2005a).

Foreign surveys performed on other members of the deer family have also demonstrated a more severe larval infestation in male animals (SAMUEL & TRAINER, 1971; BUENO-DE LA FUENTE *et al.*, 1998; VICENTE *et al.*, 2004).

3.6. Infestation indices of fawns

My investigations revealed that all infestation indices were significantly higher in case of fawns than in case of both sexes of the adult groups.

There are several possible explanations for this finding, such as the fact that fawns encounter at only several months of age swarming flies, when their defensive behavior and specific immunity are less effective.

According to SUGÁR (1978a) there are no differences between the average larval count of younger and older hosts. According to these reports, no effective protection develops in the infested hosts, therefore all age groups have the exact same chances to get infected.

Results of two other foreign surveys performed on roe deer populations have also contradicted the previously mentioned findings: the investigations of DYK & DYKOVÁ (1962) performed in Czechoslovakia revealed that the older groups (7-10 years old animals) were less frequently infested than the middle-aged and young individuals. This conclusion was drawn based on the intensity of infestation.

When investigating Czech roe deer populations, VACA (2000) found that the prevalence value of the *C. stimulator* larval infestation and the average intensity were both higher in case of one-year old animals, than in case of older hosts.

Following a survey of Hungarian red deer populations SUGÁR *et al.* (2004) did not find any differences between the prevalence values of differently aged groups, but the median intensity turned out to be higher in case of young animals (calves), than in case of the older groups.

3.7. Localization of stage one larvae

In this regard, the results of my survey were in accordance with those of DUDZIŃSKI (1970), performed on Polish roe deer populations.

According to these results, until April the larvae were localized especially in the labyrinth of the ethmoid bone. In the nasal cavities, most of the stage one larvae were found in the meatus between the ectoturbinates. A lower number of larvae were found on the mucous membrane of the endoturbinates, while the least larvae were found in the nasal conchae and choanae.

In case of the hosts dissected in April the migration of the stage one larvae toward the pharynx could be observed, in the direction of the *recessus pharyngeus*, with the purpose of positioning following molting.

3.8. Localization of stage two and three larvae

During the dissections performed in the second part of April I have also found stage two and three larvae. These were localized especially in the fossa of ROSENMÜLLER (pharyngeal recess). In case of severe larval infestations, when the pharyngeal recesses reached their maximum volumetric capacity, the larvae could also be observed attached to the choanae walls, similarly to the findings of other surveys performed on other deer species (BENNETT, 1962; DUDZIŃSKI, 1970; KENNETH, 1980; COGLEY, 1987; RUIZ *et al.*, 1993; KERTÉSZ, 1897; SUGÁR, 1974; SUGÁR, 1978a; PAPP & SZAPPANOS, 1992; MINÁŘ, 2000a).

3.9. Periods of incidence of the different larval stages

In accordance to the results of other Hungarian surveys, in case of the samples collected from October to late February and early April, only stage one larvae could be observed. In case of the samples collected in late April, stage two and three larvae were also found.

Stage two larvae could be found till late August, but their proportion showed a decreasing tendency, in contrast to stage three larvae that had an increasing ratio.

Similar observations were reported by DUDZIŃSKI (1970) and VACA (2000) following their survey on Polish and Czech roe deer populations, respectively.

3.10. Monthly dynamics of the infestation indices

During my investigations, the increasing tendency of all three major infestation indices could be observed from April to August, which is in contradiction with the findings of BARTH *et al.* (1976) and VACA (2000). The reason for this discrepancy is yet to be determined.

3.11. Effect of forest cover on botfly infestation

From the infestation indices the median intensity was significantly higher in territories with low forest cover, than in areas with high forest cover. The prevalence and average intensity values were also the highest in these habitats, but this finding could not be certified statistically. BENNETT (1962) and SAMUEL & TRAINER (1971) have also observed similar connections.

3.12. Effect of the roe deer population density on botfly infestation

From the infestation indices the prevalence and median intensity values show a statistically demonstrable relationship with the population density, hence in case of higher population densities the level infestation was also more severe. Besides these values, the average intensities did not differ.

The findings of my investigations were in accordance with the results of several other surveys (BENNETT, 1962; ALCAIDE *et al.*, 2005; FAUCHALD *et al.*, 2007).

When analyzing the parasitic infestation levels of the Hungarian roe deer populations, KUTZER *et al.* (1988) found that the infestation intensity is influenced especially by forest cover, and secondly by population density.

3.13. Effect of the host's social behavior on the parasite-host interaction

The aggregation of the parasite distribution showed more evident characteristics regarding the sexes and kids. The parasitic distribution was most aggregated in case of males (D=0.839), while the lowest value was found in case of fawns (D=0.677). In this case the prevalence of all three groups was statistically different (p=0.005), more precisely in accordance with the distribution of parasites: the prevalence value was lowest (34.60%) in case of males with the most aggregated distribution, and it was highest (54.90%) in case of fawns which had the lowest level of aggregation.

The average larval density value, used for the overall parasitic characterization did not differ in case of the populations living in forest or open habitats, but in case of the sexes and fawns the differences were statistically demonstrable. The average larval density value was the highest in case of fawns (39.36), it was lower in males (21.67) and it was the lowest in females (12.66).

Based on these values it can be concluded, that in case of fawn the larval infestation can occur more frequently, most likely due to the lack of defensive behavior of these animals and the lower resistance of their organism.

In case of males, the lower prevalence values and the more evidently aggregated distribution could be correlated with the territorial behavior of this sexual category. There are several publications that present prevalence values in case of other deer species that do not have a known territorial behavior (red deer, fallow deer, mule deer). All of these reports are present a higher and higher level of parasitic infestation of the given population. MCMAHON & BUNCH (1989) reported a prevalence of 100% in case of a North American mule deer population. In a red deer population from Spain, BUENO-DE LA FUENTE *et al.* (1998) observed a prevalence of 85%, while SUGÁR (1974; 1975; 1976 and 2004) reported a prevalence of 98.2%, 97.5%, 98.6% and 92.7% respectively in Hungarian populations. Based on the findings of my survey on roe deer populations, it can be concluded that the prevalence value in case of these territorial populations is the half or third compared to the prevalence of other, non-territorial deer species from other genera.

Based on my researches and findings of other authors, it can be concluded that the territorial behavior is a factor acting against the extensity of the parasitic infestation in the population, just like it was previously reported by RÉKÁSI *et al.* (1997) and RÓZSA (2005a; 2005b).

3.14. Effect of botfly infestation on body weight and trophy weight of roe deer

Even tough there are several reports regarding the negative consequences of botfly infestation (GRUNIN, 1957, cited by: MINÁŘ, 2000a; AGAFONOV, 1971, cited by: MINÁŘ, 2000a), the results of my survey did not sustain these observations. More precisely, I did not find any relationship between the larval intensity and eviscerated body weight, nor between the larval intensity and trophy weight.

Losses due to the parasitic infestation are caused primarily by the difficult breathing of the more severely infested individuals (MINÁŘ, 2000a), and secondly by the surplus expense of energy invested in the avoidance of the imagines (TOUPIN *et al.*, 1996; SUGÁR, 1978a).

4. Theses

1. In the Hungarian roe deer population only *Cephenemyia stimulator* (CLARK, 1815) could be found. This species can be considered universally prevalent in the Hungarian roe deer population, and its presence was recorded in 41 hunting territories of ten different counties. Based on the average of the samples collected nationwide, the prevalence of infestation of the Hungarian roe deer population was 34.6, while the average intensity was 8.87 larvae/host.

2. No statistically demonstrable difference could be observed in the main prevalence, average intensity and median intensity of botfly infestation of successive years.

3. Fawns turned out to be significantly more infested than males and females, in case of all analyzed indices (prevalence: P=0.005, average intensity: P=0.000 and median intensity: P=0.000). The explanation of this finding lies most likely in the deficient immunity. Except the average intensity (P=0,021) there were no statistically demonstrable differences between the infestation indices of adult males and females (prevalence: P=0.074, median intensity: P=0.337).

4. The increase of population density (decrease of forest cover) is in mediocre or even loose (not provable) relationship with the various botfly infestation indices. The infestation level of roe deer living in territories with a higher population density (lower forest cover ratio) was higher in case of more botfly infestation indices, than that of roe deer living in territories with low population density (higher forest cover ratio).

5. The effect of the host's social behavior on the host-parasite relationship, specifically on the average larval density value, does not result in a statistically demonstrable difference (P>0.05). Namely, there are no differences in the larval density values of roe deer populations living in forest covered or open areas, even

tough the social behavior patterns of the populations living in these two ecotypes are different.

6. The average larval density was highest in fawns (39.36). They were followed by the males (21.67), while the females had the lowest value (12.66). The aggregation (discrepancy) of larval distribution was the highest in males (0.839), it was lower in females, and it turned out to be the lowest in fawns (0.677).

7. The level of botfly infestation did not influence the body weight or the trophy weight of males.

5. Scientific publications of the thesis

Scientific publications in peer reviewed journals:

1. **KIRÁLY, I.** & EGRI, B. (2003): Az őz orr-garatbagócs fertőzöttségének 2003. évi Tolna megyei adatai. *Vadbiológia* **10**: 55-60.

2. **KIRÁLY, I.** & EGRI, B. (2004): A Tolna megyei őzállomány orrgaratbagócs-fertőzöttségéről. *Magyar Állatorvosok Lapja* **126**: 433-438.

3. **KIRÁLY, I.** & EGRI, B. (2007): Epidemiological characteristics of *Cephenemyia stimulator* (CLARK, 1815) larval infestation in european roe deer (*Capreolus capreolus*) in Hungary. *Acta Zoologica Academiae Scientiarium Hungaricae* **53**(3): 271-279.

4. MAROSÁN, M., GÁL, J. & **KIRÁLY, I.** (2002): Data relating to the body measurements of roe reer in Hungary. *Acta Agronomica Óváriensis* **44** (2): 141-147.

Congress abstracts:

5. EGRI, B. & **KIRÁLY, I.** (2002): Tolna-megyei őzek garatbagócs (*Cephenemyia stimulator* CLARK, 1818) – fertőzöttségének helyzete 2002 nyarán. *MTA Állatorvos-tudományi Bizottsága. Akadémiai beszámolók, Parazitológia és halkórtan*. **29**. p. 14.

6. MAROSÁN, M., GÁL, J. & **KIRÁLY, I.** (2003): Őzsuták testméreteinek vizsgálata. *Kárpát-medencei Biológiai Szimpózium* 2003. Budapest.

Scientific articles:

7. EGRI, B., **KIRÁLY, I.** & HÚSVÉTH, B. (2006) Hallottál már ilyet? (Vadegészségügyi széljegyzetek). *Megyei Kamarai Hírlevél.* OMVK Győr-Moson-Sopron Megyei Területi Szervezetének lapja **5**(2): 24-25.