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EPIDEMIOLOGICAL CHARACTERISTICS OF *CEPHENEMYIA STIMULATOR* (CLARK, 1815) LARVAL INFESTATION IN EUROPEAN ROE DEER (*CAPREOLUS CAPREOLUS*) IN HUNGARY

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Nasopharyngeal bot infestation in the Hungarian population of the European roe deer *(Capreolus capreolus)* was investigated between 2002 and 2005. Examination of 645 bucks, 211 does and 100 roe kids revealed that prevalence of infestation with *Cephenemyia stimulator* larvae was 34.6%. Mean intensity and median intensity of infestation were 8.9 and 5, respectively. Prevalence, mean intensity and median intensity of infestation were significantly higher in fawns compared with bucks and does. No other age-related differences were found in bucks. Out of the three larval stages, only the L1 larvae were present between October and April. From April to August the L2 and L3 larvae could be detected in the examided hosts with steadily increasing infestation indices. The L3/L2 ratio increased consistently during this period.

Key words: nasal bot fly, prevalence, intensity, roe deer, *Cephenemyia stimulator*, myiasis

INTRODUCTION

The nasal bot fly *Cephenemyia stimulator* (CLARK, 1815) is a common parasite of Roe deer throughout its distribution area. Chronobiology and descriptive epidemiology of this myasis agent have been investigated by several authors with partly unconsistent results (DUDZIŃSKI 1970, SUGÁR 1974, 1975, 1978, BARTH *et al*. 1976, NICKEL *et al*. 1986, SZAPPANOS & PAPP 1991, PAPP & SZAPPANOS 1992, LAMKA *et al*. 1997, MINAŘ 2000, MAES & BOULARD 2000, VACA 2000, KIRÁLY & EGRI 2003, 2004) (Table 1). The present survey refers to the second largest sample of roe deers of different sex and age classes ever examined in Europe for presence of the nasal bots.

Here, we address the following questions:

– Which bot fly species infest the Hungarian roe deer population?
– Which values are the most important indices of infestation (prevalence, mean intensity and median intensity)?
– How do buck infestation indices relate to different age groups?
– How do infestation indices vary among the sex and age groups?
– When do particular larval stages occur?

– What are the dynamics of prevalence, mean intensity and median intensity values?

To our best knowledge, no large-scale investigations have tested the above questions yet.

MATERIAL AND METHODS

Between 2002 and 2005, a total of 645 bucks, 211 does and 100 roe kids from 10 counties were examined. These counties are well representing the different habitats of Hungarian roe deer. Consequently, forest cover and roe population density differed across the ten counties selected. Forest cover varied between 4.3–29.8% (mean 19.7%), whereas population density varied between 2.4–4.7 specimens/km^2 (mean 3.6 specimens/km^2). The sample primarily included sport-hunted animals; thus, bot fly larvae were collected between mid April and September from the bucks and mostly between October and February from the does and kids. Bots were collected in March and early April from a few additional individuals. In the case of bucks, the so-called 'small skulls' were examined, in which a horizontal section from the dorsal corner of the protuberantia occipitalis externa to the rostral end of the nasal bone allows complete examination of the nasal and pharyngeal cavities. In the case of kids and does, the skull was split in two parts along the median plane. For 103 does and 49 kids only the presence of infestation was established, while for the remaining animals (108 does and 51 kids) all the larvae were removed and counted.

Forest cover and roe deer population density (specimens/km^2) were recorded for each county in order to test whether infestation indices covary with habitat (open vs. forest habitat) and population characteristics. Identification of the species and larval stages was carried out using a PZO MSt 130 stereomicroscope, and the keys of PAPP and SZAPPANOS (1992) and MINAŘ (2000).

Statistics were performed using the QP3.0 programme package (REICZIGEL & RÓZSA 2005). Analyses of the infestation indices of different sexes and age groups, as well as habitat and population characteristics were carried out using the χ^2 test, Fisher's exact test, Bootstrap-*t* test and Mood's median test at 95% confidence level. Habitats were classified into two types according to forest cover and population density by K-Means cluster analysis using SPSS for Windows 11.5.

Table 1. Main data of foreign and Hungarian studies dealing with nasopharyngeal bot infestation of roe deer

Author(s)	Place of study	Period	Sample number	Prevalence (%)	Mean intensity (n)
BARTH *et al*. (1976)	Germany	1972–1973	213	49*	3–11*
SUGÁR (1975, 1978)	Hungary	1974	24	70.8	12
LAMKA *et al*. (1997)	Czech Republic	1991–1995	283	60–90	no data
VACA (2000)	Czech Republic	1996–1998	1830	11.1–25.6	7.7
CURLIK *et al*. (2002)	Czech Republic	1997–1999	186	44	13
MAES & BOULARD (2000)**	France	1998–1999	68	32–43.2	no data
KIRÁLY & EGRI (2003)	Hungary	2002	143	34.8	9.8
KIRÁLY & EGRI (2004)	Hungary	2003	176	35.2	8.8

* calculated by us from the original data ; ** seroprevalence

RESULTS

The only species found was *C. stimulator*. All three larval stages were collected.

Prevalence and mean intensity in bucks from different counties are reported in Table 2. Prevalence ranged between 11.1% in Komárom (N = 9) and 76.9% in Bács-Kiskun (N = 13). Mean intensity was highest in Szolnok (19.0; N = 14) and lowest in Veszprém (3.9; N = 55). Of the sampling areas separated by cluster analysis according to forest cover three (Békés, Fejér and Szolnok counties) proved to have a low forest cover, whereas the remaining seven areas had a high forest cover. Based on final cluster centers forest cover was 7.5 % in the former, and 24.9% in the latter areas. Expressed in percentage of forest cover the distance between the cluster centers was 17.5 %. Population densities were low in four (Komárom, Somogy, Veszprém and Zala counties), and significantly higher in the remaining six sampling areas. Final cluster centers were 2.8 specimen/km^2 in the low, and 4.0 specimen/km^2 in the high population density areas. In this respect the distance between the cluster centers was 1.3 specimen/km^2. In both infestation indices higher values were consistent with higher population densities and lower forest cover. Statistically significant differences were found in the prevalence (P = 0.002) and median intensity (P = 0.026) associated with low and high population densities, whereas mean intensity was not found to differ substantially (P = 0.155). When comparing infestation indices of areas with a low vs. a high forest cover, only median intensity was found to differ significantly (P = 0.047). Differences in prevalence and mean intensity were not statistically significant (P = 0.429 and P = 0.253, respectively; Table 2).

Infestation indices were significantly higher in kids than in adult roe of both sexes (prevalence: P = 0.005 and median intensity: P = 0.000) (Table 3 & 5). On the other hand, prevalence (P = 0.457) and intensity (P = 0.289) did not differ among young, middle-aged and old bucks (Table 4). Intensity was significantly higher in bucks than in does (P = 0.016) (Table 5).

Roe examined between October and late February consistently yielded only first-stage larvae (L1). Until March, most L1 were localised in the labyrinth of the ethmoid bone, mainly the ethmoid meatus between ectoturbinates and between endoturbinates. A lower number of larvae were found on the nasal conchae and a minority in the choanae. In April, L1 had migrated towards the pharynx. Animals necropsied in the second half of April harboured 2nd and 3rd-stage larvae (L2 and L3). L2 remained detectable until August, however, their number steadily decreased during this period as opposite to L3 (Table 6 & Fig. 1). Overall, prevalence and intensity of nasal bots progessively increased from April to August (Table 7).

Table 2. Descriptors *Cephenemyia stimulator* infestations of roe-buck

County	Sample size (n)	Population density (spec./km^2)	Forest cover (%)	Prevalence (%)	Confidence interval of prevalence	Mean Intensity	Confidence interval of mean intensity	Median intensity	Confidence interval of median intensity
Baranya	21	3.7	24.3	19.00	5.44–41.91	7.75	1.25–20.00	2.0	*
Bács-Kiskun	13	3.9	19.0	76.90	46.18–94.97	8.30	4.6–12.9	5.0	2–6
Békés	49	4.6	4.3	44.90	30.66–59.77	7.77	5.41–10.32	6.5	2–11
Fejér	34	3.7	12.4	32.40	17.38–50.53	13.18	6.36–22.00	6.0	1–25
Komárom	9	2.9	26.8	11.10	0.28–48.25	15.00	*	15.0	*
Somogy	44	3.0	28.1	36.40	22.40–52.23	4.69	2.94–8.44	3.0	2–5
Szolnok	14	3.7	5.7	28.60	8.38–58.11	19.00	11.00–27.00	17.0	*
Tolna	370	4.7	17.3	37.30	32.42–42.42	9.08	7.57–11.43	5.0	5–6
Veszprém	55	2.7	29.2	16.40	7.76–28.81	3.89	1.67–9.33	2.0	1–6
Zala	38	2.4	29.9	23.70	11.44–40.25	11.33	5.22–27.56	5.0	3–14
Total	647	3.6	19.7	34.60	30.95–38.43	8.87	7.58–10.29	5.0	5–6
Low density	146	2.8	28.5	24.00	17.61–31.80	6.49	4.31–10.91	3.0	2–5
High density	501	4.1	13.8	37.70	33.53–42.11	9.31	8.05–10–95	5.0	5–6
Open habitat	97	4.0	7.5	38.10	28.79–48.44	10.59	7.76–14.49	7.0	2–11
Forest habitat	550	3.3	24.9	34.00	30.08–38.08	8.52	7.22–10.21	5.0	4–5

* sample size too small for evaluation

Table 3. Infestation indices of the roe-does and roe kids

	Sample size (n)	Prevalence (%)	CI of prevalence	Mean intensity	CI of mean intensity	Median intensity	CI of median intensity
Does	211	35.30	28.72–42.36	–	–	–	–
Kids	100	76.90	50.73–70.60	–	–	–	–
Does	108	43.50	34.00–53.40	5.94	4.57–8.40	5.0	3–6
Kids	51	54.90	40.34–68.88	24.50	17.61–32.18	20.5	8–32

Table 4. Infestation indices of roe-bucks by age group

Age group	Sample size (n)	Prevalence (%)	CI of prevalence	Mean intensity	CI of mean intensity	Median Intensity	CI of median intensity
Young	186	31.70	25.10–38.94	10.53	7.98–13.81	6.0	5–10
Middle-aged	270	37.40	31.61–43.48	8.88	7.19–11.67	5.0	4–6
Old	178	35.40	28.38–42.90	7.38	5.78–10.11	4.0	3–6

Table 5. Infestation indices of the two sexes and of the offspring

Sex/offspring	Sample size (n)	Prevalence (%)	CI of prevalence	Mean intensity	CI of mean intensity	Median Intensity	CI of median intensity
Bucks	647	34.60	30.95–38.43	8.87	7.58–10.29	5.0	5–6
Does	108	43.50	34.00–53.40	5.94	4.57–8.40	5.0	3–6
Kids	51	54.90	40.34–68.88	24.50	17.61–32.18	20.5	8–32

Table 6. Distribution of host animals and L2 and L3 larval stages by month

Month	n	L2 larval count	L2 host count	L2 %	L2 host %	L3 larval count	L3 host count	L3 %	L3 host %	Total larval count
April	5	15	4	38.5	80.0	24	5	61.5	100.0	39
May	55	54	13	17.0	23.6	264	54	83.0	98.2	318
June	27	29	10	16.3	37.0	149	26	83.7	96.3	178
July	62	100	21	14.4	33.9	595	61	85.6	98.4	695
August	75	42	18	5.6	24.0	712	75	94.4	100.0	754
Total	224	240	66	12.1	29.5	1744	221	87.9	98.7	1984

DISCUSSION

Our findings are consistent with several other studies (DUDZIŃSKI 1970, BARTH *et al*. 1976, NICKEL *et al*. 1986, SZAPPANOS & PAPP 1991, PAPP & SZAPPANOS 1992, LAMKA *et al*. 1997, MINAŘ 2000, MAES & BOULARD 2000, VACA 2000, KIRÁLY & EGRI 2003, 2004) in the sense that *Cephenemyia stimulator* was the only bot fly collected in Roe deer. Accidental infestation of a single roe deer by the red deer nasal bot, *Pharyngomyia picta* (MEIGEN, 1824) has previously been

Table 7. Monthly dynamics of infestation indices

Month	Prevalence (%)	CI of prevalence	Mean intensity	CI of mean intensity	Median intensity	CI of median intensity
April	22.7	7.8–45.4	7.8	2.8–20.0	4	1–24
May	33.3	26.2–41.1	5.8	4.3–8.8	3	3–5
June	23.9	16.4–32.8	6.6	4.4–9.6	5	1–6
July	37.8	30.4–45.7	11.2	8.5–15.0	5	3–7
August	43.4	35.9–51.1	10.1	8.2–13.3	7	5–10

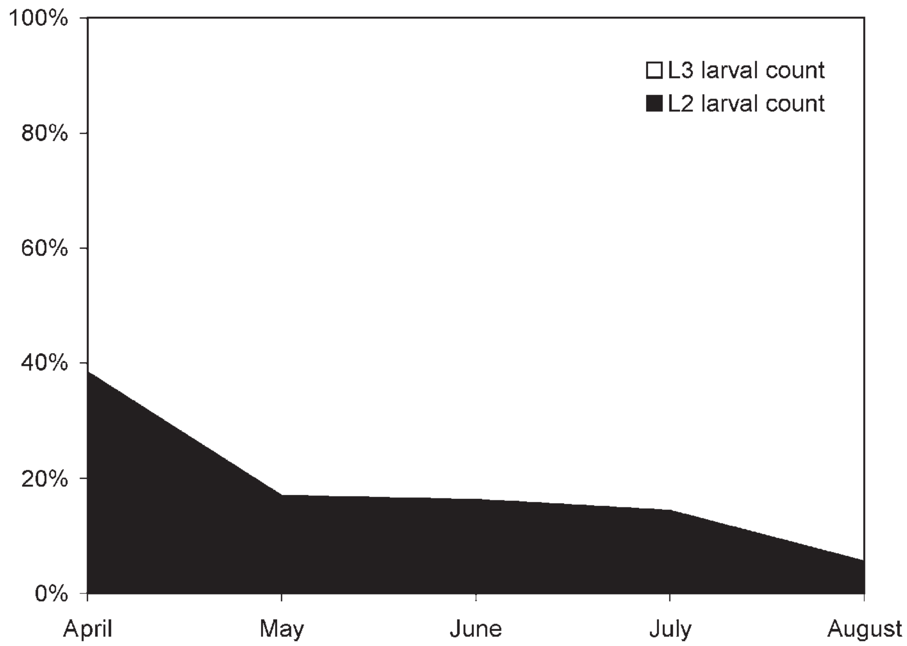

Fig. 1. Monthly dynamics of second-stage (L2) and third-stage (L3) larvae

reported by SUGÁR (1974, 1975, 1978), and *C. stimulator* was also occasionally found in red deer (KIRÁLY & EGRI 2004).

Literature data on the prevalence of nasal bots in roe deer range between a maximum of 60–90% in the Czech Republic (LAMKA *et al*. 1997) and 70.8% in a small sample in Hungary (SUGÁR 1975, 1978), and a minimum of 11.1–25.6% in the Czech Republic (VACA 2000). Our prevalence values are comprised in the above range. Similarly, intensity do not differ substantially from previously published results (DUDZIŃSKI 1970, BARTH *et al*. 1976, NICKEL *et al*. 1986, SZAPPANOS & PAPP 1991, PAPP & SZAPPANOS 1992, MINAŘ 2000, MAES & BOULARD 2000, KIRÁLY & EGRI 2003, 2004).

In this study, infestation indices were all significantly higher in fawns. This may either be attributed to the fawns' less efficient immune response, or – alternatively – to their less efficient defensive behavior against swarming flies. Higher prevalence and intensity of nasal bots in fawns are consistent with results of a previus study by KIRÁLY and EGRI (2004). Similarly, higher intensity of nasal bots in fawns than in adults was has reported in Red deer in Hungary (SUGÁR *et al*. 2004). On the contrary, nasal bot prevalence was significantly lower in fawns than in adults of Red and Fallow deer (*Cervus dama*) in Southern Spain (RUIZ *et al*. 1993). Other age-related differences in the epidemiological indices are not known in Roe deer, with the exception of a study by VACA (2000) who reported higher prevalence and mean intensity of *C. stimulator* in yearlings than in older individuals. To our best knowledge, the present study is the first to document a sex-related difference in the nasal bot infestations between Roe deer bucks and does. A recent survey of Sapnish Red deer showed that prevalence and mean intensity of oestrosis were significantly higher in stags than in hinds (BUENO-DE LA FUENTE *et al*. 1998), a result contradicting previous findings in the same host and study area (RUIZ *et al*. 1993). The authors offer several explanations for the higher infestation bucks. Briefly, bucks are believed to have a reduced resistance for a longer period of time as a result of condition loss during the mating season. Developing antlers also has high energy requirements acting against resistance. In the case of roe deer the territorial behaviour of bucks is also to be taken into account, which causes high stress and, as a consequence, energy loss.

The phenology of bot fly development in the host have already been reported by several authors. According to DUDZIŃSKI (1970), *C. stimulator* larvae can be found in the host throughout the year, L2 being present from April to July and L3 from April to August. PAPP and SZAPPANOS (1992) indicated late July as the earliest time of occurrence of L1, adding that this stage remains detectable until April or possibly May of the subsequent year. They found L2 in April and May, and L3 between April and August. However, BARTH *et al*. (1976) reported that prevalence and mean intensity of nasal bots in Roe deer in Germany tended to decrease between May and October and similar results were published by VACA (2000), whose study period extended from May to September. Findings by these authors contradict our results and, since climatic conditions in the study areas are basically similar, we are unable to explain this discrepancy.

Finally, the preferred location of first-stage larvae in the nasal cavity was consistent with DUDZIŃSKI (1970).

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