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Occurrence of *Cephenemyia stimulator* larvae in male roe deer (*Capreolus capreolus* L.) in the Lublin Upland, Poland, and their impact on particular animal health indicators

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Abstract

Introduction: The aim of the study was to carry out epizootic assessment of male roe deer to detect the presence of *Cephenemyia stimulator* larvae and determine the influence of the parasite on the carcass and antler weight in animals living in different habitats. **Material and Methods:** The investigations were based on post-mortem analysis of *Cephenemyia stimulator* infestations of the nasal passages and throat of 177 male roe deer culled between May 11 and September 30, 2020 in hunting districts of the Lublin region in Poland. The individual quality of the animals was assessed by weighing the gutted carcasses after cooling, and the antlers were weighed after dissection and their total weight was determined. **Results:** The parasite prevalence ranged from18 to 48% according to habitat type, with a mean value of 33%. The highest prevalence was detected in bucks living in grassland ecosystems. The presence of the parasites exerted influence on the individual condition of the animals, which was reflected in reduced carcass and antler weight. The differences were found both in young individuals and in somatically fully developed animals, but they were not statistically significant in all age groups. **Conclusion:** Although the parasitic infestations impaired the individual condition traits, their parametric values in most cases were not lower than those reported from other regions of Poland. Despite the *Cephenemyia stimulator* infestation, male roe deer from the Lublin region are characterised by high carcass and antler weight.

Keywords: roe deer, parasites, *Capreolus capreolus*, *Cephenemyia stimulator*.

Introduction

Roe deer (*Capreolus capreolus*) is the most numerous game species living in Poland. It has adapted to living in a variety of habitats, but the contact zones between plant communities, referred to as ecotones, are the optimal environments for the species (18). In western Poland, a field ecotype of roe deer preferring vast agrocenosis areas and avoiding forests almost completely has additionally evolved. Due to the variety of roe deer habitats, these animals are exposed to multiple diseases of varied aetiology. Parasitic diseases are one of the most important threats. While the available literature provides ample information on endoparasites attacking tissues and organs of animals from various regions of Poland (7, 17), there are few reports on the occurrence of *Cephenemyia* fly larvae. There are also no data on their influence on the animal health status, which is an indicator of the state of the animal's health and the environmental pressure it has experienced. The pathogenic effect of parasites, in addition to deprivation of nutrients for the host through competition, may also be mechanical damage to tissues and organs leading to haemorrhages and sometimes intestinal or bronchial obstruction. Parasites secrete toxic metabolic products in the host organism, which can trigger numerous allergies contributing to the development of the conditions of weakness, emaciation, and cachexia. They also have an impact on animal behaviour and may lead to the death of severely infested hosts. In male deer, they may affect the annual cycles of antler growth, and thus considerably affect their

development and form and the animal's body weight (20, 29). More than 60 species of parasites attacking various tissues and organs have been found to date in Polish cervids (9, 16). The symptoms and effects of *Cephenemyia* infestations on the host organism depend mainly on the age of the host and the number of invading larvae (27). Although the disease caused by botfly larvae located in the nasal cavities and throat may affect all representatives of the Cervidae in Poland, there are few reports on roe deer in this respect. Investigations conducted near Kraków showed a 13% rate of infestation of the roe deer population with varied prevalence of the parasite (16).

The parasite has been found in roe deer for many years not only in Poland but also in other countries, and its prevalence has been seen to depend mainly on the local population density (3, 22, 23, 28). In 2012, an infestation by the larvae of this fly was diagnosed for the first time in two male roe deer in Extremadura in Spain, which is the southern most region of occurrence of this parasite (6). However, the research in this field is still insufficient, and there are no detailed data on the presence of the parasite in Poland or other European countries.

Due to the habitat and climate diversity of different regions of Poland, roe deer are characterised by high variability in their carcass weight and/or antler weight and form. Roe deer from the Lublin region have substantially higher carcass and antler weight than those from other areas in Poland (12, 14, 32, 34). The presence of *Cephenemyia* flies weakens physiological processes in the infested animals; therefore, parasite infestations affect the condition of individual male roe deer, which is reflected in carcass and antler weight, common indicators of the quality of cervid males as hunt prey. The hypothesis about the differences in the individual quality of animals being predicated on the presence or absence of parasites was verified in roe deer from three habitats varying in the density of these botflies. The aim of the study was to assess the scale of *Cephenemyia stimulator* infestations and the effect of these parasites on the quality of male roe deer harvested in the Lublin region, collating data from three different habitats of the culled animals.

Material and Methods

The study was based on post-mortem analysis of the occurrence of *Cephenemyia stimulator* invasion in the nasopharyngeal cavity of male roe deer culled by hunters between May 11 and September 30, 2020, *i.e.* in the legal hunting season for this species and sex (13).

Animals. Animals intended for the tests were delivered to the collection centre by hunters from the appointed districts in the Lublin region. The roe deer originated from three different habitats: agricultural land (agroecosystems), grassland environments, and forest areas. In total, 177 animals were assessed, of which 60 originated from agroecosystems, 60 from grassland ecosystems, and 57 from forest ecosystems. The analysed sample should be regarded as representative, as it originated from random culling of animals respecting the hunting intensity and selection criteria for individual male roe deer specified under Polish hunting law. The animals were culled during one hunting season. Thus, the environmental pressure in terms of trophic and climatic conditions which directly affect the animal condition and physiological processes related to antler growth was comparable for every deer.

The skin, muscles, and connective tissue as well as the eyes and brain were removed from the animal's head, which ensured access to all potential locations of the parasitic larvae (Fig. 1). During these procedures, the throat and oesophagus were assessed as well. Where *Cephenemyia* spp. presence was seen, the larvae of the parasite in individual animals were counted (Fig. 2). During the analyses, the carcass was weighed. Age was also determined by assessment of changes in the infundibulum of mandibular premolars and molars, exploiting method is widely applied in hunting practice and scientific research (26). After dissection, the antlers were weighed.

Fig. 1 *Cephenemyia stimulator* larvae in the nasal passages and throat of a culled male roe deer

Fig. 2. *Cephenemyia stimulator* larvae collected for assessment and counting

Statistical analyses. The carcass and antler weights were analysed statistically to calculate mean values and standard error of the mean. A two-way analysis of variance was performed, taking into account the age and habitat of the animals. The Newman–Keuls test in Statistica 10.0 (StatSoft, Tulsa, OK, USA) was used to compare the means. Obtained results are

summarised in tables that show the number of infected and non-infected animals (n), the percentage of infected animals to the total number of animals from a given ecosystem (%), as well as the mean value and the minimum and maximum values of a given feature. The comparison included mean carcass weight and antler weight values between infected and noninfected individuals within the individual ecosystems, as well as between the individual ecosystems. The comparisons were made within age groups and for the entire sample regardless of animal age.

Results

Older animals were found to be infested by clearly the largest number of parasitic larvae (Table 1). In 2- and 3-year-old individuals, from 1.6 to 4 parasites were found on average, with the maximum being 7. The mean number of parasites in older bucks increased with age, and the highest number (n = 14) was found in 5-year-old animals from grassland habitats. Animals culled in field ecosystems were infested by the highest number of larvae, followed by individuals from grassland habitats, and in those from forest ecosystems the lowest number of larvae was found. The parasite prevalence varied depending on the animal habitat. The lowest infestation prevalence of 18% was determined in bucks from forest ecosystems, whereas the highest level of 48% was detected in animals culled in grassland habitats. Overall and not differentiating by age or habitat, parasite prevalence was estimated at 33%.

The mean carcass weights of the culled bucks varied with the animal's age. In almost all cases, infested animal carcasses weighed less (Table 2). Statistically significant differences between the carcass weights of infested and non-infested animals were found in the group of 6-year-old and older bucks from agroecosystems. The mean difference amounted to 2.65 kg. Similar differences were also found in the group of 4-year-old animals from grassland habitats and forest ecosystems. When the bucks were not stratified by age, there were statistically significant differences between the infested and non-infested only in animals from agroecosystems.

The infestation by the *Cephenemyia stimulator* parasite contributed to differences in antler weight (Table 3). Statistically significant differences in this trait were found in 2-year-old bucks from agroecosystems. The mean difference amounted to 68 g. Non-infested animals from grassland ecosystems were characterised by substantially higher antler weight in all age groups except the youngest bucks. These differences were statistically significant in all cases. Similarly, non-infested bucks from forest ecosystems were characterised by higher antler weight in all age groups, but statistically significant differences were found only in 4- and 5-year-old individuals.

Hierarchical ordering of the deer by carcass and antler weight indicates a clear dominance of individuals from agricultural ecosystems. Regarding the carcass weight trait, bucks from agroecosystems were the heaviest and the difference was statistically significant compared to the carcass weight of bucks from forest ecosystems. The highest weight of antlers was also recorded in roe from agroecosystems, but no statistically significant differences were found in this parameter between the regions of origin.

Table 1. Number of confirmed *Cephenemyia stimulator* parasites in male roe deer presented age and habitat

Age		Habitat (ecosystem)		
		Agro	Grassland	Forest
2	n (%)	5 (33.3)	3 (21.4)	1 (7.1)
	mean	1.6	1.7	1
	min	1.0	1.0	1
	max	2.0	3.0	1
3	n (%)	4 (36.3)	6 (50.0)	3 (27.3)
	mean	4.0	3.5	2.3
	min	2.0	1.0	1.0
	max	7.0	7.0	3.0
4	n (%)	1 (10.0)	8 (61.5)	2 (20.0)
	mean	1	5.9	4.5
	min	1	2.0	4.0
	max	1	12.0	5.0
5	n (%)	3 (27.3)	4 (40.0)	3 (30.0)
	mean	7.0	6.3	3.3
	min	5.0	2.0	2.0
	max	11.0	14.0	5.0
≥6	n (%)	7 (53.8)	8 (72.7)	2 (16.7)
	mean	6.0	4.0	2.5
	min	2.0	1.0	2.0
	max	12.0	7.0	3.0

Table 2. Male roe deer carcass weight presented habitat and presence of *Cephenemyia stimulator* parasites

Habitat (ecosystem)	Item		Age (years)					Total/Mean
			2	3	4	5	≥6	
Agro	Non-infested	n	10	7	9	8	6	40
		mean	15.85	17.92^{a}	19.22	20.18^{a}	21.00^{a}	18.61^{a}
	Infested	n	5	4	1	3	7	20
		mean	14.40	15.87^{b}	17.62	18.16^{b}	18.35^{b}	16.88^{b}
	Mean		15.37^{x}	17.18	18.87^{xy}	19.64^{x}	19.57	18.02^{x}
	SEM		0.92	0.69	1.24	1.06	1.41	1.12
Grassland	Non-infested	n	11	6	5	6	3	31
		mean	14.63	17.75^{a}	20.40^{a}	20.16^{a}	20.00^{a}	17.75
	Infested	n	3	6	8	4	8	29
		mean	15.00	15.91^{b}	17.12^{b}	18.25^{b}	18.50^{b}	17.18
	Mean		14.71^{xy}	16.82	18.38^{x}	19.40^{x}	18.91	17.48^{xy}
	SEM		0.73	0.89	0.92	0.86	0.72	0.27
Forest	Non-infested	n	13	8	8	7	10	46
		mean	14.23	16.81^{a}	18.25^{a}	18.00	18.95	16.97
	Infested	n	1	3	2	3	2	11
		mean	13.84	15.66^{b}	16.75^{b}	17.16	19.00	16.48
	Mean		14.22^{y}	16.50	17.9550^{y}	17.75^{y}	18.96	16.86^{y}
	SEM		0.65	0.87	0.53	0.81	1.42	2.10

SEM – standard error of the mean; a, b – mean values of carcass weight of infested and non-infested individuals within an ecosystem marked with different letters differ significantly at $P \leq 0.05$; x, y – mean values of carcass weight of individuals from different ecosystems marked with different letters differ significantly at $P \leq 0.05$

Table 3. Gross antler weight of male roe deer presented by habitat and presence of *Cephenemyia stimulator* parasites

Habitat (ecosystem)	Item		Age (years)					Total/Mean
			2	3	4	5	≥6	
Agro	Non-infested	n	10	7	9	8	6	40
		mean	189.20^{a}	287.42	401.33^{a}	419.25	419.16	334.62
	Infested	n	5	4	1	3	7	20
		mean	121.20^{b}	238.75	331.12^{b}	368.33	388.14	283.31
	Mean		166.53^{x}	269.73	394.31^{x}	405.36	402.46	318.10
	SEM		20.54	16.25	31.24	25.44	17.14	18.12
Grassland	Non-infested	n	11	6	5	6	3	31
		mean	163.27	294.66^{a}	404.60^{a}	421.33^{a}	480.00^{a}	308.22
	Infested	n	3	6	8	4	8	29
		mean	155.00	223.66^{b}	323.00^{b}	327.50^{b}	398.37^{b}	306.48
	Mean		161.50^{x}	259.17	354.38^{xy}	383.80	420.64	307.38
	SEM		18.89	34.12	36.44	34.88	39.78	38.47
Forest	Non-infested	n	13	8	8	7	10	46
		mean	161.69	265.50	327.25^{a}	379.57^{a}	409.00	295.46
	Infested	n	1	3	2	3	2	11
		mean	153.24	236.33	246.00^{b}	319.00^{b}	390.00	293.80
	Mean		137.16^{y}	257.55	311.00^{y}	361.40	405.83	295.16
	SEM		34.80	20.94	22.12	12.44	16.14	22.84

SEM – standard error of the mean; a, b – mean values of carcass weight of infested and non-infested individuals within an ecosystem marked with different letters differ significantly at $P \leq 0.05$; x, y – mean values of carcass weight of individuals from different ecosystems marked with different letters differ significantly at $P \leq 0.05$

Discussion

Roe deer are exposed to parasitic infestations in their living habitats. In Poland, a relatively large number of investigations have focused on the occurrence of endoparasites in the respiratory and digestive systems (8, 15, 24, 25, 31). Similar studies have also been conducted in other European countries (19, 35, 36). The results of research carried out in Portugal showed that nearly half of culled roe deer were infested with at least one parasite (11), and in Sweden, parasitic infestations were responsible for 10% of roe deer mortality (1).

Although studies on the occurrence of endoparasites in roe deer indicate the scale of the problem, infestations of cervid species by *Cephenemyia stimulator* flies are rather poorly described (3, 16, 22, 28). The disease caused by botfly larvae located in the nasopharyngeal cavity in horses can occur in all

representatives of the Cervidae family, but there are few scientific reports on this problem in roe deer. A 13% rate of infestation with varied prevalence of the parasite was reported in a population of roe deer living near Kraków (16). In turn, as shown in a study from Spain, 23.8% of analysed roe deer exhibited the presence of the parasite (21). Another study from Spain conducted to identify the most appropriate antigen in the serodiagnosis of *Cephenemyia* infestations showed average infestation intensity of 24.3 larvae per infested animal from a group of 43 roe deer (2).

There are almost no reports on the impact of this parasite on the condition of animals. Respiratory and nutritional disorders usually leading to severe emaciation are mainly reported. There are also disorders in the change of the hair coat or in velvet loss (27). The comparison of the carcass weights of bucks analysed in the present study with those of male roe deer culled in the same area at the beginning of the 2000s showed for the carcasses from 2020 higher weight of non-infested and lower weight infested male roe deer from field and grassland environments (10). Similarly, lower carcass weight was determined in all individuals from forest habitats in the present study compared to animals culled in the early 2000s. However, antler weight was higher in the present study than that noted in the 2000s, regardless of the presence of parasitic infestation and the living environment of the animals (10). In comparison with data from 2008 and 2010 on bucks from field and grassland habitats, carcass weight in non-infested animals was higher, whereas in infested animals it was lower. Irrespective of the presence of infestation, carcass weight in bucks from forest ecosystems was lower. In comparison with the results from 2008 and 2010, antler weight was higher in non-infested and lower in infested individuals, regardless of the habitat (12).

Carcass and antler weights in the infested and non-infested bucks harvested in the Lublin region were substantially higher than in the case of roe deer culled in south-western Poland (near Opole) in the 2012/13 hunting season (34). These parameters also exceeded those reported for bucks culled in the surroundings of Kraków, especially in older age groups (33). The present values were higher in both groups (infested/non-infested), especially in the case of older animals, than those determined by Wajdzik *et al.* (32) in male roe deer living in various habitats in the Kielce region. Concurrently, antler weight was substantially higher in non-infested bucks but similar to the values reported from the Kielce region in 2010–2012 in infested males (32). Carcass weights of roe deer culled in 2005–2011 in the foothills of the Eastern Sudetes indicate the parameter to be clearly higher in the non-infested male roe deer from the Lublin region analysed in the present study. Similarly, carcass weights in the infested animal group, regardless of the habitat, were slightly higher on average than in the case of the roe bucks from the Eastern Sudetes (30). Despite the differences between the study areas, the average carcass weight of bucks culled in northern Poland in 2011–2004 was lower than that of infested and non-infested bucks analysed in the present study (14).

The results of the study of *Cephenemyia stimulator* infestation of roe deer indicate that parasite prevalence depended on the type of habitat and the age of the animals. The highest infestation level was found in grassland habitats, and the lowest in forest ecosystems. The overall roe deer infestation rate was 33%, which was similar to results reported from other European countries. Parasites were directly responsible for the decline in animal carcass weight, which was unmistakeable in the infested animals, and statistically significant differences were found between some age groups in all types of habitat. The differences between animals from older age groups were smaller and statistically insignificant, which may imply that older animals cope better with environmental pressure, including parasitic infestations. Non-infested animals from grassland ecosystems of all ages past 2 years old had substantially higher antler weight, and the differences were statistically significant. In the case of bucks harvested in forest ecosystems, there were also differences between all age groups as well, and the highest statistically significantly different values were determined in 4- and 5-year-old males. Further research in this field and development of prophylaxis against infestations by the larvae of this insect are worthwhile to undertake.

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