Nasopharyngeal bot flies in red deer (*Cervus elaphus*) from southern Spain

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M. Gonzálvez, C. Martínez-Carrasco*, F. Alonso, L. León & R. Ruiz de Ybáñez

Department of Animal Health, Faculty of Veterinary Science, Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, 30100 Murcia, Spain

*Corresponding author: cmcpleit@um.es

Summary

The nasopharyngeal bot flies *Pharyngomyia picta* and *Cephenemyia auribarbis* (Diptera: Oestridae) are parasites that have an impact on the health of wild ruminants. Little is known about their biological and epidemiological characteristics in multi-host habitats such as Cazorla Natural Park (CNP), in southeast Spain. This paper describes the main features of bot fly larvae parasitising the population of red deer in CNP. The results reveal an overall bot fly prevalence of 37.5%, with a *P. picta* and *C. auribarbis* co-infection rate of 12.5%. Although the statistical analyses were not significant ($p > 0.05$), a higher prevalence in males and in calves was observed, which has to be explained from a multifactorial viewpoint. Unfavourable climatic conditions during January induced the overwintering of larvae inside the host and, accordingly, a higher prevalence of the first instar (L1) was detected, while higher prevalence of L2 and L3 was recorded in February and March. Further studies are needed to investigate in more detail the environmental characteristics that influence the chronobiology of bot flies in southeast Spain.

Keywords

Cephenemyia auribarbis – Cervid – Iberian Peninsula – Oestridae – *Pharyngomyia picta*.

Introduction

Nasopharyngeal myiasis is a condition of domestic and wild ruminants which mainly affects the respiratory capacity of animals and reduces the host's body condition. Despite their epidemiological relevance, bot flies have been rarely studied (1, 2, 3, 4). Different species of the subfamily Oestrinae (Diptera: Oestridae) are present in the Holarctic region and parasitise several species of wild ruminants (4, 5, 6, 7, 8, 9, 10, 11, 12). Two important members of this subfamily are *Cephenemyia auribarbis* and *Pharyngomyia picta*, which are host-specific obligate parasites of wild ruminants such as those belonging to the genera *Cervus*, *Dama* and *Capreolus* for *P. picta*, and *Cervus* and *Dama* for *C. auribarbis* (9, 13). Both species have been frequently reported as a cause of nasopharyngeal myasis in cervids in America and Europe, including red deer (*Cervus elaphus*) from different Spanish regions (4, 7, 9, 14, 15). In addition, it is commonly accepted that *P. picta* and *C. auribarbis* usually co-infect red deer in Spain (6, 7, 14).

The larviposition of first stage larvae (L1) by adult bot flies is carried out in the nostrils, or on the upper lip or lower part of the muzzle of hosts, causing stress to the animals (9, 14). Moreover, the location of larvae in the tracheopharyngeal region of the host provokes lesions on tissues and, consequently, rhinitis, sinusitis, sneezing, nasal discharge, dyspnoea, difficulty swallowing, weakness, asthenia and decrease of body condition (2, 10). In severe cases, a high number of larvae can lead to host death (16).

The population of red deer has increased during recent decades in the Iberian Peninsula, mainly influenced by a greater availability of resources, implementation of rigorous hunting schedules, supplementary feeding and the underestimation of deer populations in spring (17, 18, 19). In fact, Spain is one of the European countries with the highest populations of red deer (19).

The Sierra de Cazorla, Segura y Las Villas Natural Park (hereinafter CNP) is located in Jaén province (Andalusia, Spain), and is one of the most important European protected areas owing to its size (214,300 ha) and to the variety of habitats that it covers. In addition, four species of wild ruminants coexist in the park: the Iberian ibex (*Capra pyrenaica*), European mouflon (*Ovis aries musimon*), red deer and fallow deer (*Dama dama*), the last two being natural hosts for nasal bot flies. These environmental characteristics make CNP an area of special interest for studying the epidemiological features of these bot flies in the southeast of the Iberian Peninsula. In order to improve the health management of wildlife species, it is necessary to obtain as much information as possible about parasite–host interactions, expanding from the one-host–one-parasite framework to complex dynamics of multi-host communities (20). Thus, the objectives of this study were *1)* to determine the prevalence and the intensity of *P. picta* and *C. auribarbis* and *2)* to investigate the relationship of nasopharyngeal myiasis with biotic and abiotic factors in the population of red deer in CNP.

Materials and methods

Cazorla Natural Park has a Mediterranean and continental climate, mainly conditioned by altitude (between 450 and 2,100 m above sea level) (6). The mean rainfall and temperature values (range) recorded during the study period were 61.5 mm (0–174.2) and 8.8°C (0–20), respectively. Climatic data corresponded to registers in Torre Vinagre Station and were provided by the State Meteorological Agency upon request.

A total of 64 red deer were harvested in CNP (59º54′N, 17º51′W) between November and April of 2003, 2004 and 2005. The number of animals collected during each sampling month varied from five to eighteen deer. Animals were recovered during the hunting periods of the above-mentioned years, as well as in application of the population management programmes established by the direction of the CNP. Deer were individually registered, including information about the location, sex and age of each animal. All the animals showed good body condition and were classified as calves (<12 months old) or adults (≥1 year old) according to the evaluation of the age based on their dentition (21). Finally, 36 females and 28 males, including 55 adults and 9 juveniles, underwent post-mortem examination. This information is summarised in Table I.

Table I

Prevalence and median intensity of bot fly larvae in red deer from Cazorla Natural Park

By sex, month and age category of the host (calves: <12 months; adults: ≥1 year); *n* = number of animals; prevalence = percentage of deer infected; median intensity = number of larvae per infected deer

		Age		Sex		Month					
	Total deer	Calves	Adults	Male	Female	November	December	January	February	March	April
Prevalence (95% CI)	37.5% ± 11	55.6% ± 15.4	34.5% ± 7.5	50% ± 12.8	30% ± 9.2	0%	0%	37.5% ± 2.6	47% ± 6	50% ± 13.3	44.4% ± 8.5
Median intensity (range)	9 (1–34)	9 (3–13)	9 (1–34)	8 (1–16)	10 (1–34)	–	–	10 (1–34)	8 (2–16)	9 (1–18)	10 (2–34)
n	64	9	55	28	36	7	5	8	17	18	9

CI: confidence interval

Post-mortem examinations were performed in the field. First, the head of each deer was separated from the body, making a sagittal cut with an axe; subsequently, the oropharynx, oesophagus and trachea were opened longitudinally, in order to make a careful macroscopic evaluation of the nasopharyngeal cavity, paranasal sinuses and adjacent tissues to detect larvae of bot flies. Any lesion was noted and evaluated in detail. Isolated larvae were preserved in 10% formaldehyde and sent to the Department of Animal Health (University of Murcia, Spain) for morphometric identification. The general shape, morphometric features, distribution of rows of spines and pigmentation were used to identify larval species and stages. Moreover, all larvae were cleared (10% potassium hydroxide, 24 h), dissected and mounted in Hoyer's medium to evaluate the posterior spiracles and buccal hooks under a microscope (22). For the identification of bot fly larvae, it is relevant to note that the use of molecular techniques for larval identification shows analogous results to those obtained with the morphometric methodology (23). All sample collection and parasite identification were performed by experts in parasitology from the Veterinary Faculty of the University of Murcia, Spain.

The prevalence of larvae was estimated from the ratio of positive animals to the total number of animals tested. The number of larvae per animal showed a non-normal distribution according to the evaluation of skewness (D'Agostino skewness test), kurtosis (Bonett–Seier test) and the Shapiro–Wilk normality test. Consequently, the prevalence of larvae was compared among groups using Pearson's chi-squared test or Fisher's exact test, as appropriate, and the total number of larvae and instar larvae – L1, L2 and L3 – using non-parametric tests (Wilcoxon signed-rank test and Kruskal–Wallis test). Explanatory variables were the host's sex and age, sampling month and geographical animal distribution. To test the relationships of prevalence and parasite intensity between bot fly larvae species, the Fisher's exact test and Spearman's rank correlation, respectively, were used. Significance was assessed with alpha = 5% ($p < 0.05$), and R software v3.6.0 (24) was used for all these analyses.

Results

A total of 254 bot fly larvae were isolated during this study. Among these larvae, 94.5% of Diptera specimens were identified as *P. picta* ($n = 240$ larvae collected from 24 deer) and 5.5% as *C. auribarbis* ($n = 14$ larvae collected from eight deer) according to their morphometric characteristics. The overall prevalence of infection with the 95% confidence interval (CI) was 37.5 ± 11%, considering that *P. picta* showed a prevalence of 37.5% (24/64) and *C. auribarbis* of 12.5% (8/64). Co-infection with *P. picta* and *C. auribarbis* larvae was detected in 12.5% of the studied deer (i.e. one third of the positive red deer). Regarding the intensity of infection, the total median intensity (and range) was 9 (1–34) larvae per deer, with 8 (1–34) larvae corresponding to *P. picta* and 1 (1–5) to *C. auribarbis*.

Considering the prevalence of nasopharyngeal larvae in the deer, without taking into account the species of Diptera to which they belonged, it was found that the monthly and bimonthly prevalence values were not statistically different ($p > 0.05$). However, the values revealed a rising prevalence (± 95% CI) from November and December (0%) to March (50 ± 13.3%), followed by a slight decrease in April (44.4 ± 8.5%), when prevalence was evaluated monthly. In turn, median intensity and its range showed the same trend, although the records maintained very similar values from January (10 larvae; 1–34) to April (10 larvae; 2–34) after the period of absence of larvae from November to December. Statistical analysis of the temporal dynamics of bot fly median intensity did not show significant differences among months ($p > 0.05$).

The median intensity of both nasopharyngeal larval species throughout the study showed similar values in all sex and age categories, as well as when the geographical distribution of animals was evaluated ($p > 0.05$), although the prevalence of bot flies in males and calves tended to be higher than in females and adults ($p > 0.05$) (Table I). Larvae at stage L1 showed a prevalence of 10.9 ± 2.3% and an intensity of 1 (1–9), L2 of 17.2 ± 9.2% and 2 (1–5), and L3 of 35.9 ± 5.9% and 8 (1–32). Stage L1 larvae were present (n given for *P. picta/C. auribarbis*) only in January ($n = 5/0$), February ($n = 1/0$) and March ($n = 1/0$). Stage L2 larvae were detected in January ($n = 2/0$), February ($n = 12/0$), March ($n = 2/2$) and April ($n = 3/0$) and L3 were collected in January ($n = 10/0$), February ($n = 71/4$), March ($n = 82/5$) and April ($n = 51/3$).

The prevalence of *P. picta* was significantly higher than that of *C. auribarbis* and the intensity of *P. picta* was positively correlated with that of *C. auribarbis* (Fisher's exact test, $p = 0.00017$, risk index = 3.4, and Spearman's rank correlation, $p = 1.67e–05$, rho [correlation coefficient] = 0.5, respectively).

Discussion

Although infections with bot flies have traditionally been undervalued in wild ruminant species, previous authors have highlighted the importance of *P. picta* and *C. auribarbis* because they can produce impaired ventilation capacity and deterioration of the body condition, growth and fat reserves of red deer by limiting the intake of forage (3, 4).

Previous studies showed a prevalence of *P. picta* and *C. auribarbis* over 81% in red deer and fallow deer in southern Spain between 1992 and 1996 (6, 14). However, surveys carried out in red deer in central Spain between 1990 and 2003 described a similar prevalence of these bot fly infections to that of this study (37.5%) (3, 4). Small climatic variations predetermine the epidemiology of the whole Order Diptera by affecting flies' behaviour, such as mating activity, survival and consequent larviposition by adult oestrid females (25, 26). Concretely, the strong influence of climatic conditions on the life cycle of both nasopharyngeal bot fly species has previously been described (4, 9). On the other hand, individual host factors (e.g. pregnancy, fitness) may also be involved in the prevalence of bot fly larvae registered because these variables can modulate the immune status (9).

Co-infection with *P. picta* and *C. auribarbis* was found in 12.5% of the evaluated CNP red deer, despite *P. picta* being much more prevalent (94.5%) than *C. auribarbis* (5.5%). These values are lower than those previously described in the south of the Iberian Peninsula, in which co-infection was seen in 23–74% of parasitised red deer (4, 6, 7, 14). Concerning the monthly distribution of prevalence and parasite intensity, the current study showed differences in maximum and minimum values when compared with surveys carried out in southern and central Spain (3, 4, 14). This discrepancy in the results suggests that it is necessary to deepen the study of environmental factors in order to determine whether the existence of microclimates could be the cause of these fluctuations in oestrid prevalence over years and locations. Other variables such as host species diversity and their abundance may also be influencing the occurrence and temporal distribution of bot fly larvae.

The statistically significant differences detected in the prevalence and parasite intensity between the two nasopharyngeal larval species are similar to the results obtained by Vicente *et al.* (4). This finding suggests that animals suffering from co-infection may be the individuals in the deer population most susceptible to being parasitised by both species of nasopharyngeal larvae, indicating that parasitic aggregation exists, as has been suggested in previous studies (4). These interactions have been described not only between Diptera species but also between Diptera (Syrphidae) and Coleoptera (Cetoniidae) (27). Nevertheless, other variables including individual characteristics and environmental factors should be considered.

This study showed that male deer tend to have a higher prevalence of bot fly larvae than females, in agreement with the results of previous studies (4, 14). This could be associated with a decrease in immune status as a consequence of the stress to which males are subjected during some periods of the year, especially during and after the rutting season (winter and spring), as has been described to occur with different parasite infections in ungulates (28, 29, 30). In this sense, the immunosuppressive effect of testosterone has a negative influence on the immune system and favours parasite development (28). Also, the activity patterns, the use of habitat and the differential behaviour between sexes when they are attacked by flies for larviposition could influence the differences in prevalence among hosts (14, 31). Moreover, the wider retropharyngeal cavity of males may favour the presence of oestrids, in comparison with females (14). According to the results of this study, there were no statistically significant differences in the prevalence detected by age ($p > 0.05$), nor in the mean intensity ($p > 0.05$) by sex and age of the deer. These results could be a consequence of the low and partially efficient immune response that has been described in ruminants after repeated exposures to cavitary myiasis (32), which allows animals to suffer repeated infections year after year.

The prevalence of larvae of all instars isolated in this study follows the same pattern as results previously outlined in southern Spain (14). The greater presence of L1 in January, coinciding with the lowest average temperature recorded in the period of study (–3.5°C), seems to indicate overwintering of this stage within the host's nasopharyngeal cavity (9). The absence of *C. auribarbis* larvae in January may be due to the asynchrony described between the biological cycles of *P. picta* and *C. auribarbis*; this could be considered as a mechanism to avoid the overlap of the two species, which seems to help mainly *P. picta* (14). However, the low prevalence of *C. auribarbis* in the red deer population studied should be taken into account when interpreting these data. Our results suggest that abiotic factors, such as temperature and precipitation, may regulate the life cycle of bot flies, and may even be able to prevent their development in certain microclimates, as described for other bot fly species (9, 33). However, additional surveys including environmental and biotic factors are needed to better understand the epidemiological characteristics of myasis in CNP.

As future challenges, further studies on bot fly in red deer will be necessary to provide new information on the epidemiology of *P. picta* and *C. auribarbis*, mainly regarding the parasite intensity according to the sex and age of the host, the annual distribution of these Diptera, and the influence that the presence of sympatric species, such as red deer and fallow deer, may have on the epidemiology of myiasis in Mediterranean areas.

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