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Species composition of *Gasterophilus* spp. (Diptera, Oestridae) causing equine gastric myiasis in southern Italy: Parasite biodiversity and risks for extinction

Domenico Otranto [a,*], Piermarino Milillo [a], Gioia Capelli [b], Douglas D. Colwell [c]

[a] *Department of Animal Health and Welfare, Faculty of Veterinary Medicine, University of Bari, Valenzano, Bari, Italy*
[b] *Istituto Sperimentele delle Venezie, Legnaro, Padua, Italy*
[c] *Agriculture & Agri-Food Canada, Lethbridge Research Centre, Canada*

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Abstract

Horse gastrointestinal myiasis caused by larvae of *Gasterophilus* spp. (Diptera, Oestridae) flies has a worldwide distribution and, where present, it is primarily caused by larvae of *Gasterophilus intestinalis* and *Gasterophilus nasalis*. Other species, i.e., *Gasterophilus inermis*, *Gasterophilus pecorum* and *Gasterophilus haemorrhoidalis*, present in different or in the same regions of the gastrointestinal tract, were only occasionally reported in very limited areas of eastern European Countries and in central Italy. With the aim to contribute data on the species composition of *Gasterophilus* and on the seasonal variation of the infection pattern in southern Italy, 152 native horses were necropsied from January to December 2003 and *Gasterophilus* larvae were collected from the stomach, the small intestine and the rectum of each of them.

On the whole, 125 (82.2%) horses were infected by larvae of *Gasterophilus* spp. and 214 second stage larvae (L 2) and 13,342 third stage larvae (L3) collected. Five species of *Gasterophilus* were identified with the following prevalence: *G. intestinalis* = 95.2%, *G. nasalis* = 44.8%, *G. inermis* = 15.2%, *G. pecorum* = 2.6% and *G. haemorrhoidalis* = 0.8%. L3 were retrieved throughout the observation period with the highest mean burdens from January to August 2003 while the lowest mean was registered from September to November 2003. L2 were collected in February–March 2003 and from September to December 2003. The majority of the animals ($n = 66$, 43.4%) were infected with a single *Gasterophilus* species, however, 45 animals (29.6%) harboured 2 species, 12 animals (7.9%) 3 species and 2 animals (1.3%) 4 species. The trend of abundance in the L3 of *G. intestinalis* and *G. nasalis*, the most represented species, was highly concordant ($r = 0.5$, $p < 0.001$). A retrospective comparison of our results and of other data from four seasons of observation (1983–1986) in central Italy showed that the number of *G. inermis*, *G. pecorum* and *G. haemorrhoidalis* have been

* Corresponding author. Tel.: +39 080 4679839; fax: +39 080 4679839.
E-mail address: d.otranto@veterinaria.uniba.it (D. Otranto).

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decreasing relative to *G. intestinalis* and *G. nasalis* which may suggest tendency toward the extinction of the three former species of *Gasterophilus*.

Keywords: Myiasis; *Gasterophilus intestinalis*; *Gasterophilus nasalis*; *Gasterophilus inermis*; *Gasterophilus pecorum*; *Gasterophilus haemorrhoidalis*; Horse

1. Introduction

The genus *Gasterophilus* (Diptera, Oestridae) includes eight species of flies whose larvae cause gastrointestinal myiasis in equids. These larvae are present, for about 10 months, in different regions of the equid gastrointestinal tract (g.t.) (Zumpt, 1965). *Gasterophilus intestinalis* (De Geer, 1776) and *Gasterophilus nasalis* (Linnaeus, 1758) are distributed worldwide and are often the only species reported in many parts of the New World (Zumpt, 1965; Schooley et al., 1971; Kettle, 1974; Drudge et al., 1975; Lyons et al., 1994). *Gasterophilus inermis* (Brauer, 1858), *Gasterophilus pecorum* (Fabricius, 1794) and *Gasterophilus haemorrhoidalis* (Linnaeus, 1958) are only reported in very limited areas of Europe and Eastern Countries (Zumpt, 1965; Egri et al., 1995). All five species of *Gasterophilus*, mentioned above, have been reported in horses from Umbria, central Italy (Principato, 1989), often occurring simultaneously.

Since larvae of different species of *Gasterophilus* are specifically present in one or more regions of the g.t., the pathology they induce should depend by the species involved. Generically, gasterophilosis is characterized by difficulties in swallowing (throat localization of the immature stages), gastro and intestinal ulcerations, gut obstructions or volvolus, rectal prolapses, anaemia, diarrhoea and digestive disorders (Waddell, 1972; Dart et al., 1987; Principato, 1988; Cogley and Cogley, 1999; Sandin et al., 1999; Sequeira et al., 2001). In any case, difficulties in estimating and quantifying the mechanical injuries and physiological damages caused by gasterophilosis, may explain the fact that this myiasis is often underestimated and less studied than other myiases of livestock (e.g., cattle hypodermosis and sheep oestrosis).

In addition to their significance as parasites of horses, there are reports of human myiasis associated with *Gasterophilus* spp. (Anderson, 2005). Subcutaneous-creeping or opthalmo-myiasis by *Gasterophilus* spp. first stage larvae (L1) have been reported (James, 1947). Infection by second stage larvae (L2) has also been recorded in an infant (Royce et al., 1999).

In Italy gasterophilosis has been studied in northern (Poglayen and Zoffoli, 1976) and central Italy (Principato, 1987, 1989) although few data are available for southern Italy and the islands (Puccini et al., 1988).

The composition of *Gasterophilus* species and their abundance during the year is of importance not only in the planning of control programs and for reporting of clinical signs of pathologies in equids, but it is also of interest from a biological and ecological point of view.

The aim of this work is to contribute data on the species composition of *Gasterophilus* and on the seasonal variation of the infection pattern in autochthonous horses over 1 year of grazing season in southern Italy. The importance of biodiversity in species composition of *Gasterophilus* is also discussed in view of the extinction of some of these species.

2. Materials and methods

2.1. Study area and necropsy procedures

From January to December 2003, 152 native horses were necropsied at a slaughterhouse in Noicattaro municipality (province of Bari, Apulia region, south-eastern Italy) (latitude 39° and 41° North, longitude 15° and 18° East). The locality where these animals originated is characterized by a Mediterranean-type climate with cool winters, rainfall between October and April and a hot, dry summer.

Necropsies were carried out once a week (about 2–3 animals per week) only on autochthonows animals traditionally managed and reared under extensive conditions in the Apulia region. Animals imported from Eastern Countries (e.g., Romania, Poland, Czech

Republic), which constituted the highest part of slaughtered horses, were excluded. The age and sex of each animal were recorded together with information about farm location and anti-parasitic treatments.

After a first observation to retrieve any ectopic extra-intestinal larval migration, the g.t. was stretched out and the esophagus, the stomach, small intestine (i.e., the duodenum about 50 cm from the pylorus), the large intestine (i.e., cecum and rectum) were isolated using a string.

The different regions were then cut longitudinally and when present, larvae were removed, and placed into vials containing water. Larvae were than delivered to the Parasitology Unit of the Veterinary Medicine, Faculty of Bari University for morphological identification.

2.2. Identification procedures

All the larvae collected were washed in saline (NaCl 0.9%) and identified using a stereomicroscope (LEICA MS5) with a magnification capacity from 12 to 80. Morphological identification was based on the description of Zumpt (1965).

2.3. Statistical analysis

Data were summarized in terms of prevalence, abundance and intensity of infection according to Bush et al. (1997). Differences among prevalence in relation to season, sex and age classes were tested by the Chi square test (EPI.INFO 6.0) and the differences were considered to be significant when $p \leq 0.05$. Correlation of L2 and third stage larvae (L3) of each *Gasterophilus* species were tested by Spearman rank correlation. Software used was SPSS for Windows, Version 12.0, and the differences were considered to be significant when $p \leq 0.05$.

3. Results

No anti-parasitic treatments were administrated to the examined animals. The overall prevalence of infection with *Gasterophilus* spp. larvae was 82.2% (125 of 152). The number and percentage of positive and negative horses, grouped according to sex and age, are reported in Table 1. A total of 214 L2 and 13, 342 L3 were collected with a mean intensity of 108.4 larvae (Table 2). No significant differences were registered in both prevalence and mean larval burden among horses of different age or gender ($p > 0.05$).

Table 1
Number and percentage of positive and negative horses grouped according to sex and age

	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Horses	125	82.23	27	17.76	152	
Sex						
Male	57	79.16	15	20.83	72	47.36
Female	68	85	12	15	80	52.63
Age						
<1 year	96	78.68	26	21.31	122	80.26
1–2 year	22	95.65	1	4.34	23	15.13
>2 year	7	100	–	–	7	4.60

Gasterophilus intestinalis was the most common species (95.2%) followed by *G. nasalis* (44.8%), *G. inermis* (15.2%), *G. pecorum* (2.6%) and *G. haemorrhoidalis* (0.8%) (Table 3). The species and the number of *Gasterophilus* recovered, along with the months they were found in the horses are reported in Table 2.

L3 were retrieved throughout the observation period with the highest mean burden from January to August 2003 while the lowest mean was registered from September to November 2003 (Fig. 1). L2 were collected in February–March 2003 (n. 9) and from September to December 2003 (n. 205) (Fig. 1).

The majority of the animals were infected by 1 (66–43.4%) or 2 (45–29.6%) *Gasterophilus* species, 12 animals (7.9%) by 3 species and 2 animals (1.3%) by 4. The maximum number of larvae retrieved in the same horse was noted in April when 738 L3 were counted in a single animal (i.e., 689 *G. intestinalis* in the stomach, 39 *G. nasalis* in the duodenum and 10 *G. inermis* in the rectum).

Ulcers and erosions were found in all the animals and in each district of the g.t. where bots were found attached to the mucosa. All the *G. intestinalis* and *G. pecorum* larvae were recovered from the oesophageal region of the stomach. *Gasterophilus nasalis* were recovered from the first portion of the duodenum and *G. inermis* from the rectum. The two specimens of

Table 2
Monthly prevalence of *Gasterophilus* spp. larvae: number and percentage of horses found to be positive, number of larvae collected (divided according their stage, second L2-stages and third L3-stages) and mean larval burden

Month (2003)	No. examined horses	No. positive horses (%)	Number of larvae			Mean larval burden
			L2	L3	Tot	
January	20	19 (95)	–	1324	1324	90.73
February	12	10 (83.33)	2	723	725	72.50
March	21	16 (76.19)	7	2097	2104	131.5
April	13	12 (92.30)	–	2549	2549	212.41
May	13	12 (92.30)	–	1727	1727	143.91
June	9	7 (77.77)	–	1367	1367	195.28
July	12	9 (75)	–	383	383	42.55
August	3	3 (100)	–	834	834	278
September	15	8 (53.33)	17	106	123	15.37
October	12	8 (66.66)	12	292	304	38
November	12	11 (91.66)	74	304	378	34.36
December	10	10 (100)	102	1636	1738	173.8
Total	152	125 (82.23)	214	13342	13556	108.44

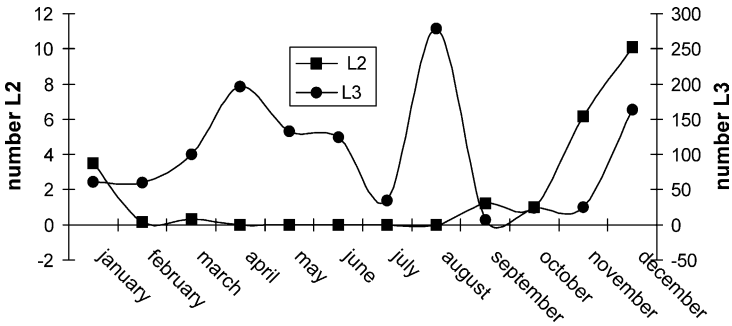

Fig. 1. Abundance of L2 and L3 during the year.

G. haemorrhoidalis were found in the rectum of a horse in the August 2003.

Seasonality of L3 for each *Gasterophilus* species (Table 4) is shown in Fig. 2. The trend of abundance in the L3 of *G. intestinalis*, *G. nasalis*, the most common and abundant represented species, was highly concordant ($r = 0.5$, $p < 0.001$). Significant correlation was also found between L3 of *G. intestinalis* and L3 of *G. nasalis*, L2 of *G. inermis* and L3 of *G. pecorum* ($r = 0.5$, $p < 0.001$), L3 of *G. nasalis* and L3 of *G. inermis* ($r = 0.29$, $p < 0.001$) and L3 of *G. intestinalis* and L3 of *G. inermis* ($r = 0.27$; $p < 0.001$).

Table 3
Number and percentage of *Gasterophilus intestinalis*, *Gasterophilus nasalis*, *Gasterophilus inermis*, *Gasterophilus pecorum* and *Gasterophilus haemorrhoidalis* in the horses examined and number of larvae collected for each species

	Horses infected		Larval number	
	No.	%	No.	%
G. intestinalis	119	95.2	11282	83.22
G. nasalis	56	44.8	1891	13.94
G. inermis	19	15.2	319	2.35
G. pecorum	4	2.6	62	0.45
G. haemorrhoidalis	1	0.8	2	0.01

4. Discussion

The high prevalence of *Gasterophilus* spp. larvae (82.23%) in the present study is similar to that previously reported from horses in Italy (94%) (Principato, 1989) and from donkeys, in other countries of the Mediterranean basin (e.g., Morocco,

Table 4
Number of infected horses broken down according to the different species of *Gasterophilus* and relative density (r.d. calculated as mean number of larvae ± d.s.) divided according months of collection

Month	*G. intestinalis*			*G. nasalis*			*G. inermis*			*G. pecorum*		
	Horses infected	No. of larvae	r.d.	Horses infected	No. of larvae	r.d.	Horses infected	No. of larvae	r.d.	Horses infected	No. of larvae	r.d.
January	19	863	45.4 ± 31.5	9	422	46.8 ± 44.2	3	38	13 ± 0.6	1	1	1 ± 0
February	10	689	68.9 ± 85.5	2	36	18 ± 24	–	–	–	–	–	–
March	16	1685	105.3 ± 82.8	10	284	28.4 ± 24.2	5	80	16 ± 16.9	2	55	27.5 ± 29
April	11	2334	212.1 ± 202.1	4	142	35.5 ± 28.5	3	73	24.3 ± 12.4	–	–	–
May	12	1460	121.6 ± 73.8	7	262	37.4 ± 44.6	1	5	5 ± 0	–	–	–
June	8	1092	136.5 ± 74.7	6	264	44 ± 27.1	2	11	5.5 ± 0.7	–	–	–
July	8	308	38.5 ± 44	2	69	34.5 ± 31.2	–	–	–	1	6	6 ± 0
August	3	792	264.6 ± 250.8	2	40	20 ± 24	–	–	–	–	–	–
September	7	115	16.4 ± 11.8	2	8	4 ± 1.4	–	–	–	–	–	–
October	5	243	48.6 ± 31.9	5	61	12.2 ± 5.6	–	–	–	–	–	–
November	10	323	32.3 ± 28.7	2	35	17.5 ± 13.4	1	20	20 ± 0	–	–	–
December	10	1378	137.8 ± 98.3	5	268	53.6 ± 60.1	4	92	23 ± 24.9	–	–	–
Total	119	11282	94.8	56	1891	33.76	19	319	16.7	4	62	15.5

Pandey et al., 1992; Jordan, Mukbel et al., 2001). On the whole, the prevalence of gasterophilosis is higher than reported in England and Wales—53% (Edwards, 1982), Germany and Ireland—43% (Bauer, 1986; Sweeney, 1990) and in Sweden—9.9% (Hoglund et al., 1997). The differences may be explained by management factors (i.e., grazing animals are left untreated in southern Italy) and by the favorable climatic and ecological conditions of Italy for the development, maintenance and transmission of gasterophilosis (see below).

With regard to the seasonality of the infection by different species of *Gasterophilus*, it is known that development from egg to L2 requires approximately six weeks while L3 develop for about 10 months in the host g.t. (Soulsby, 1982). In the present survey, L3 of *G. intestinalis* and *G. nasalis* were found throughout the observation period, with the lowest mean intensity of L3 from September to November 2003 for both species and the highest mean intensities from January to August 2003. Interestingly, the bimodal intensities of L3 of *G. intestinalis* in April and August may suggest the presence of a long period of fly activity with two or more cohorts of flies active during the previous summer and autumn. The later peak of *G. intestinalis* L3 may be also explained by a pupal over

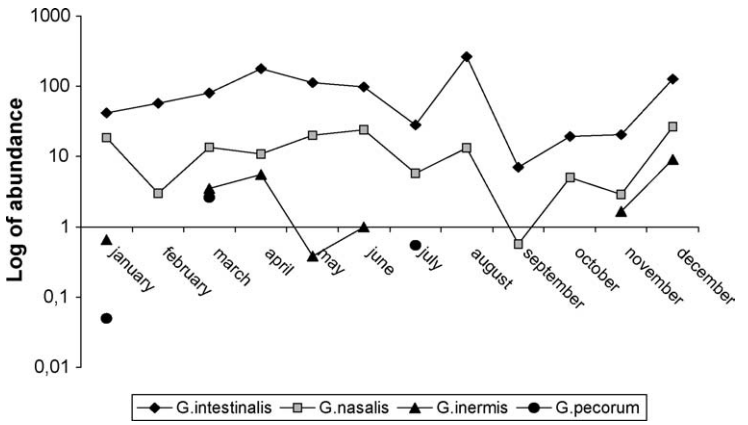

Fig. 2. Seasonality of third stage larvae of *Gasterophilus* species.

wintering phenomenon. However, the presence of L2 only from September to December indicates it is unlikely that more than one infection cycle occurs in the same season as reported, in southern Italy, for other species of Oestridae causing myiasis (i.e., the sheep nasal botfly *Oestrus ovis*).

In central Italy, the highest incidence of *G. intestinalis* and *G. nasalis* L3 was noted in February–March with a decrease in the following months leading to a complete absence from the beginning of August (Principato, 1989). In northern European Countries (i.e., in UK and Sweden), L1 of *G. intestinalis* were detected in September (indicating summer fly activity) but no L3 were found until October (Edwards, 1982; Hoglund et al., 1997).

Our finding of *G. nasalis* L3 throughout the year is similar to that registered in Morocco in donkeys (Pandey et al., 1992) and in Kentucky (Drudge et al., 1975) and the presence of L2 from January to March 2003 and in December of the same year indicates a peak of fly activity in autumn, as for *G. intestinalis*. The absence of *G. inermis* L3 from July to October is consistent with data registered, for the same species, in central Italy (Principato et al., 1984) and indicates fly activity in the late summer.

Although gasterophilosis appears to be well tolerated by horses, it is difficult to believe that massive infections (up to 689 L3 in the stomach, 39 in the duodenum and 10 in the rectum of the same horse) do not cause direct damage to the g.t. and to the digestive processes. On the contrary, it is reasonable to assess that the total effect of extensive pitting by a large number of larvae is harmful to horses and impairs animal productivity and welfare by changing the digestibility of food. Another aspect that has to be taken into account is the role of the mucosal lesions caused by *Gasterophilus* L3 in the occurrence of equine colic (Hatch et al., 1976). This suggests that the impact of this myiasis is probably underestimated because of difficulties in quantifying the damage it causes.

Co-occurrence of five different species of *Gasterophilus* affecting horses in southern Italy is of ecological interest as it represents the highest biodiversity of this genus at any locality. Gasterophilosis probably originated in Eurasian regions and spread, together with horses, to many parts of the world (Zumpt, 1965). This hypothesis is supported by the fact that in many parts of the New World (e.g., United States and in New Zealand) only a few species such as *G. intestinalis* and *G. nasalis* are present (Schooley et al., 1971; Kettle, 1974) while in very limited areas of Europe and Eastern Countries the presence of *G. inermis*, *G. pecorum* and *G. haemorrhoidalis* has been reported (Zumpt, 1965; Principato et al., 1984).

The species composition of *Gasterophilus* may be affected by the host (e.g., genetic differences, race susceptibility), by the parasite (e.g., genetic differences, population composition) and by the environmental and management factors (e.g., pharmaceutical treatments, different animal husbandry, orography of the area, plants cultures). On the whole, *G. intestinalis* and *G. nasalis* are the predominant species (reviewed by Mukbel et al., 2001). In particular, the high prevalence of *G. intestinalis* worldwide and its success, in terms of occurrence with respect to other species, may be a result of the fact that *G. intestinalis* develops in the stomach where there is a large habitat for larval growth. At this site, the larvae have more abundant resources than the other species (e.g., those living in the duodenum and the rectum) and consequently, the adults are more vigorous and have a higher biotic potential.

Since data on prevalence of different species of *Gasterophilus* in the past are lacking from southern Italy, the results of the present study have been compared with the only data on the species composition of *Gasterophilus*, available in Central Italy (Principato, 1989). This comparison was made on the basis of the closeness of the two regions (Apulia and Umbria) in which observations were carried out.

Principato (1989) reported that during four seasons of observation (1983–1986) the number of *G. inermis*, *G. pecorum* and *G. haemorrhoidalis* decreased relative to *G. intestinalis* and *G. nasalis* species. Our work confirms this trend and, in particular, the presence of only two specimens of *G. haemorrhoidalis* throughout the observation period indicates a likely tendency toward the extinction of this species of *Gasterophilus*.

Furthermore, the data reported in the present study indicate that southern Italy plays an important role for the maintenance of several species of myiasis causing larvae whose existence is endangered by the modern

livestock production system (confined breeding, pharmaceutical treatments).

For example, *O. ovis* and *Hypoderma lineatum* are still present in many Countries of the Mediterranean Basin, while they have almost disappeared from other regions. Other species such as *Przhevalskiana silenus* (causing goat warble fly infection) and *Rhinoestrus* spp. occur in small foci within Europe. *Przhevalskiana silenus* is largely confined to the Apulia and Calabria regions (Otranto and Puccini, 2000) and *Rhinoestrus* spp. has been recently reported for the first time in Apulia and Sicily (Otranto et al., 2004). The role of southern Italy in the conservation of parasite biodiversity may be explained by:

(1) the geographical position that was and still is a hub of commercial pathways for animals from the Eastern (Albania, Greece, Poland, Romania, Russia, etc.), Southern (Morocco, Egypt, Algeria), Western (Spain, France) and Northern countries (through northern Italy transit animals from the northern Europe). The fact that animals imported from Eastern countries (e.g., Romania, Poland, Czech Republic) constitute the highest part of slaughtered horses support this hypothesis;
(2) the management is often constituted by free grazing animals that remain untreated thus harbouring a number of endo- and ecto-parasites;
(3) ecological factors (extensive pastures are shared by animals of different farms);
(4) the Mediterranean climate that is favourable to the development of the biological cycle of several myiasis causing oestrids.

Without doubt, broad spectrum anti-parasitic treatments, (e.g., macrocyclic lactones) constitute a critical factor for the selection of species of *Gasterophilus* and for the maintenance of their biodiversity in a given area. This phenomenon has been also compounded by the reduction of the number of other equids (e.g., donkeys) that act as reservoirs to harbour species of *Gasterophilus*.

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