Bull. Soc. Pathol. Exot. (2018) 111:132-138
DOI 10.3166/bspe-2018-0013

Report on *Gasterophilus* spp. (Diptera, Gasterophilidae) of Horses in Algeria: Prevalence, Intensity, and Monthly Variations

Infection par *Gasterophilus spp*. (Diptera, Gasterophilidae) chez des chevaux en Algérie : prévalence, intensité et variations mensuelles

D.E. Kraouchi · S. Meradi · B. Bentounsi

Received: 10 November 2017; Accepted: 19 February 2018

Abstract The composition, prevalence, and seasonal dynamic of *Gasterophilus* species were studied at the slaughterhouse of Constantine region (East Algeria) in 128 horses over an 18 months period. Our survey revealed that 124 (96.9%) horses were infected with a mean intensity of 161 larvae of *Gasterophilus* spp. Four species of *Gasterophilus* were identified; *Gasterophilus intestinalis* and *G. nasalis* were by far the predominant species with 95.3% and 77.3% respectively, followed by *G. haemorrhoidalis* (14.0%) and *G. pecorum* (10.1%). The age, sex, and breed of horses did not affect the distribution of the infection. Second-stage larvae (L2) of *G. intestinalis* were absent between April and July and present in higher numbers between December and February. In addition, third-stage larvae (L3) were few in number, mainly from September to November, a consequence of their fecal elimination and suggesting that the effective period of adult activity of *G. intestinalis* is autumn. Therefore, the population dynamics of *G. nasalis* shows that its activity is two months longer and occurs earlier than that of *G. intestinalis*. The incidence of *G. pecorum* infection was linked to the rainiest months.

Keywords Gasterophilosis · Gasterophilus intestinalis · G. nasalis · G. haemorrhoidalis · G. pecorum · Horse · Prevalence · Seasonality · Slaughterhouse · Constantine · Algeria · Maghreb · Northern Africa

Résumé La composition, la prévalence et la dynamique saisonnière des espèces de *Gasterophilus* (ou gastérophiles) ont été étudiées à l'abattoir de la région de Constantine (est de l'Algérie) chez 128 chevaux sur une période de 18 mois. L'enquête a révélé que 124 (96,9 %) chevaux étaient infectés avec une intensité moyenne de 161 larves de *Gasterophilus spp*. Quatre espèces de *Gasterophilus* ont été identifiées. *Gasterophilus intestinalis* et *G. nasalis* étaient de loin les espèces prédominantes avec respectivement 95,3 % et 77,3 %, suivis de *G. haemorrhoidalis* (14,0 %) et de *G. pecorum* (10,1 %). L'âge, le sexe et la race des chevaux n'ont pas affecté la répartition de l'infection. Les L2 de *G. intestinalis* étaient absentes entre avril et juillet et présentes en nombre maximum entre décembre et février. De plus, les L3 étaient peu nombreuses principalement de septembre à novembre, une conséquence de leur élimination fécale, ce qui suggère que la période effective d'activité adulte de *G. intestinalis* est l'automne. La dynamique de population de *G. nasalis a* montré que son activité était plus longue de deux mois et se produisait plus tôt que celle de *G. intestinalis*. L'incidence de l'infection à *G. pecorum* était liée aux mois les plus pluvieux.

Mots clés Gasterophilose · Gasterophilus intestinalis · G. nasalis · G. haemorrhoidalis · G. pecorum · Cheval · Prévalence · Saisonnalité · Abattoir · Constantine · Algérie · Maghreb · Afrique du Nord

Introduction

More than 150 species of internal parasites infect horses [7]. *Gasterophilus spp*. are obligate parasites commonly found in the gastrointestinal tract of equids, and affecting the horses' health by absorbing nutrients and secreting toxins [36]. An unusual finding of larval *Gasterophilus intestinalis* deeply inserted in the diaphragmatic muscle was recently reported [5]. Infection by *Gasterophilus spp*. induces generically non-specific clinical signs such as difficulties in swallowing

D.E. Kraouchi · B. Bentounsi (✉)
Institute of Veterinary Sciences, Constantine University 1, 25000 Constantine, Algeria
e-mail : bbentounsi@umc.edu.dz

S. Meradi
Institute of Veterinary and Agronomic Sciences, Batna University 1, 05000 Batna Algeria

(throat localization of the larvae), gastric and intestinal ulcerations, gut obstructions or volvulus, rectal prolapses, anemia, and diarrhea [9,10,19,36]. In addition to their significance as parasites of horses, there are reports of human myiasis associated with *Gasterophilus spp*. causing subcutaneous creeping or ophtalmo-myiasis [2,14,30]. Nine species of *Gasterophilus* (Diptera, Gasterophilidae) flies have been described. While *Gasterophilus intestinalis* and *Gasterophilus nasalis* are distributed worldwide, the remaining seven species are usually reported in limited areas of Eastern Europe and Africa [22]. The presence of *Gasterophilus* species has been investigated in different countries such as Belgium, Ireland, China, Germany, Kazakhstan, and Poland, with rates varying between 43 and 100% [1,13,15,18,29,33]. In Algeria, no data on the prevalence and on the species composition of *Gasterophilus spp*. are available. The horse breeding areas have been located in some sites since the colonial period. The breeds of horses in Algeria are dominated by the autochthonous Barb, the thoroughbred Arab, and several crosses (mainly Arab–Barb). Equine parasite infections have been poorly investigated in Algeria. Aside from one study describing the incidence of strongyle infections [4], the prevalence and distribution of the other parasite species in Algerian horses remain largely unknown. Therefore, the current work aims to provide more insights on the epidemiology of equine parasites in the eastern part of Algeria by investigating the prevalence and species fauna of *Gasterophilus*. It also aims to determine the monthly variations of the infection pattern among three kinds of Algerian horses during a one year and a half period.

Materials and methods

Study area and necropsy procedures

The study was carried out during an 18 month period in a slaughterhouse on the occasion of sanitary inspections of consumer meat. This slaughterhouse is located in Khroub (East Algeria), which is situated 80 km south of the Mediterranean coast, 635 m above sea level. The climate is Mediterranean semi-arid [6], with a hot dry summer extending from June to August and a wet cold period from September to March–April. The average annual rainfall is 630 mm. January is the rainiest month with an average rainfall of 94 mm.

The necropsied horses (n=128) were aged between 8 and 15 years. Seven horses were of the autochthonous Barb breed, 35 were identified as Arab–Barb crossbreeds, and the majority (88 horses) was unidentified crossbreeds. The horses' population was composed of 99 males and 29 females. After a first observation to retrieve any ectopic extra-intestinal larvae migration, the stomach, small intestine (i.e., the duodenum about 50cm from the pylorus), the large intestine (i.e., cecum and rectum) were isolated using a string. The different regions were cut longitudinally and when present, larvae were removed and placed in water.

Morphological identification was carried out in the Parasitology Unit of the Institute of Veterinary Sciences, Constantine University. No information was available on the medical history of the horses, pasture or anti-parasitic treatment, nor on the actual place in the country where they were bred.

Identification procedures

All the collected larvae were washed in saline solution (NaCl 0.9%) and identified using a stereomicroscope with a magnification capacity ranging from 12 to 80. Morphological identification was based on the description of Zumpt [37].

Statistical analysis

A Chi square test was performed to establish differences of infection between factors: age, sex, and breed. The differences were considered to be significant when the p-value was ≤ 0.05 (significance threshold). Monthly dynamics of each *Gasterophilus* species (based on mean number of the larvae) were analyzed using an analysis of variance (ANOVA) test, followed by a post hoc Tukey test to classify the months into high or low infection periods. All the statistical analyses were performed using SPSS 20 software.

Results

The overall prevalence of infection with *Gasterophilus spp*. larvae was 96.9% (124 of 128 horses). The number and percentage of positive and negative horses, grouped according to age, sex, and breed, are reported in Table 1. No significant differences were registered in terms of prevalence among horses of different age, sex, or breed ($p > 0.05$).

A total of 2074 second-stage larvae (L2) and 17,825 third-stage larvae (L3) were collected with a mean intensity of 160 larvae. *Gasterophilus intestinalis* was by far the most common species (95.3%) followed by *G. nasalis* (77.3%), *G. haemorrhoidalis* (14.0%), and *G. pecorum* (10.1%). During the first year of the study, the ANOVA analysis showed that the dynamic of the mean larval burden was statistically different throughout the months studied ($p = 0.03$). The period from January to July showed the highest mean burden ($p = 0.02$), while the lowest mean was registered from September to November ($p = 0.05$). During the semester monitored in the second year, no differences in the mean larvae burden were recorded ($p = 0.27$) (Table 2). During the first year, the lowest number of L3 was recorded from September to November. L2 were collected in the first year from September until January, and February of the second year.

Table 1 Number and percentage of horses tested positive and negative with *Gasterophilus* spp. larvae grouped according to age, sex, and breed in eastern Algeria / *Nombre et pourcentage de chevaux positifs et négatifs par Gasterophilus spp. dans l'est de l'Algérie, les larves sont regroupées selon l'âge, le sexe et la race*

	Positive		Negative		Total		*p*-value of Chi^2 test
	N	%	*N*	%	*N*	%	
Horses	124	96.9	04	3	128	100	
Age							
< 3 years	54	96.4	2	3.6	56	45.3	$p = 0.08$
3 to 8 years	52	100	0	0	52	39.1	
> 8 years	18	90	2	10	20	15.6	
Sex							
Male	95	96	04	4	99	77.3	$p = 0.27$
Female	29	100	0	0	29	22.7	
Breed							
Barb	7	100	0	0	7	5.5	$p = 0.87$
Arab–barb	34	97	1	2.9	35	27.3	
Unidentified crossbreeds	83	96.5	3	3.5	86	67.2	

Table 2 Monthly variations of *Gasterophilus* spp. larvae: number and percentage of horses found to be positive, number of larvae (L2 and L3) collected and mean larval burden in eastern Algeria / *Variations mensuelles des larves de Gasterophilus spp. : nombre et pourcentage de chevaux trouvés positifs, nombre de larves (L2 et L3) collectées et charge larvaire moyenne dans l'est de l'Algérie*

	No. Examined Horses	No. Positive Horses (%)	Number of larvae			Mean larval burden
			L2	L3	Total	
First year						
January	10	9 (90)	175	1329	1504	167
February	12	11 (92)	0	1983	1983	180
March	6	6 (100)	42	780	822	137
April	10	10 (100)	0	2381	2381	238
May	2	2 (100)	0	760	760	380
June	5	5 (100)	0	780	780	156
July	5	5 (100)	5	1369	1374	275
September	10	9 (90)	92	606	698	78
October	10	10 (100)	482	473	955	96
November	9	8 (89)	32	527	559	70
December	10	10 (100)	621	1328	1949	195
Total first year	89	85 (95.5)	1449	12316	13765	162
Second year						
January	7	7 (100)	240	655	895	128
February	5	5 (100)	186	434	620	124
March	5	5 (100)	21	434	455	91
April	4	4 (100)	41	634	675	169
May	9	9 (100)	137	1445	1582	176
June	9	9 (100)	0	1907	1907	212
Total second year	39	39 (100)	625	5509	6134	150
Total	128	124 (96.9)	2074	17825	19899	161

Half of the animals (n = 71, 55.5%) harbored two *Gasterophilus* species; 29 animals (22.6%) were infected by a single *Gasterophilus* species; 22 animals (17.1%) by three species; and two animals (1.6%) by four species. The seasonality of the infection by different species of *Gasterophilus* is shown in Table 3.

L3 of *G. intestinalis* and *G. nasalis* occurred in all months of the year. Those of *G. pecorum* were prevalent in November to February and those of *G. haemorrhoidalis* in January to May. However, L2 were absent from April to July for *G. intestinalis*, and June and July for *G. nasalis*. No L2 were found for the two other species.

Discussion

The high prevalence of *Gasterophilus* spp. larvae (96.9%) in the present study was similar to that previously reported from horses in Italy (94%) [28] and from donkeys in other countries of the Mediterranean basin such as Morocco [25] and Jordan, [21]. On the other hand, the prevalence of gasterophilosis is higher than reported in England and Wales (53%) [8], Poland (47%) [31], Germany and Ireland (43%) [3,35], and Sweden (10%) [12]. The differences may be due to various factors including grooming of animals during the period of fly activity, the number of horses, the presence of donkeys and mules in a given area increasing the probability of cross-infection, and the favorable Mediterranean climate. This difference might also be explained by the fact that grazing animals are often left untreated in Algeria. These results suggest that both *G. intestinalis* and *G. nasalis* are the predominant species in Algeria. Similar results were reported in other countries in the Mediterranean zone, such as in Morocco [24], in Italy [23,27], and in Jordan [21].

The prevalence of *G. intestinalis* is comparable to those described in New Zealand [16], Ireland [35], and the United States of America (USA) [26], where the infection rates varied between 90 and 100%. In addition, the high prevalence of *G. intestinalis* worldwide may be due to the fact that this parasite 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 [23].

The prevalence of *G. nasalis* in Algeria in this sample is higher than in other countries. In New Zealand, Kettle [16]

Table 3 Monthly variations of prevalence, number of larvae (L2 and L3) of infected horses according to the mean number of different species of *Gasterophilus* larvae in eastern Algeria / *Variations mensuelles de la prévalence, nombre moyen de larves (L2 et L3) des chevaux infectés selon les espèces de Gasterophilus dans l'est de l'Algérie*

	G. intestinalis			***G. nasalis***			***G. pecorum***			***G. haemorrhoidalis***		
	Horses infected (%)	**No L2**	**No L3**	**Horses infected (%)**	**No L2**	**No L3**	**Horses infected (%)**	**No L2**	**No L3**	**Horses infected (%)**	**No L2**	**No L3**
First year												
January	9 (90)	15.9	125.9	0	0	0	2 (20)	0	3.36	0	0	0
February	11 (91.7)	0	104.7	8 (66.7)	0.3	45.2	5 (41.7)	0	8.45	5 (41.7)	0	15.4
March	6 (100)	5.7	92.3	4 (66.7)	0.7	37.7	0	0	0	0	0	0
April	10 (100)	0	177.4	9 (90)	1	58	0	0	0	3 (30)	0	2.7
May	2 (100)	0	342	2 (100)	0.4	26	0	0	0	2 (100)	0	12
June	5 (100)	0	148.6	2 (40)	0	7.4	0	0	0	0	0	0
July	5 (100)	0	263.6	5 (100)	0	10.2	0	0	0	0	0	0
September	8 (80)	3.18	16.5	9 (90)	5.18	38.5	0	0	0	0	0	0
October	5 (50)	3.90	0.2	8 (80)	44.3	24.3	0	0	0	3 (30)	0	2.8
November	8 (88.9)	1.75	39.9	6 (66.7)	2.25	25.8	2 (22.2)	0	0.2	0	0	0
December	10 (100)	60.4	69.8	7 (70)	1.7	52.4	3 (30)	0	1.8	2 (20)	0	8.8
Second year												
January	7 (100)	35.6	52.3	6 (85.7)	0.3	40.8	1 (14.3)	0	1	3 (42.9)	0.4	1
February	5 (100)	33.6	63.4	5 (100)	1.6	23.4	0	0	0	0	0	0
March	5 (100)	1.4	58.8	5 (100)	2.8	27.8	0	0	0	0	0	0
April	4 (100)	0	102.7	4 (100)	5	55.7	0	0	0	0	0	0
May	9 (100)	0	83.7	8 (88.9)	1.5	76.7	0	0	0	0	0	0
June	9 (100)	0	190.8	8 (88.9)	0	21	0	0	0	0	0	0

found them in 59% of horses. In the USA, Panitz [26] reported that the incidence was even lower with 30%, while in Ireland, *G. nasalis* was slightly higher with 65% of infected animals [35]. All these values should be considered with the knowledge if the animals are treated or not.

The low prevalence of species other than *G. intestinalis* and *G. nasalis* can be explained on the one hand by a likely tendency toward an extinction of these species of *Gasterophilus*, in agreement with Otranto et al. [23], and on the other by their persistence in sites where the pressure of competition is lower. This latter is suggested by the fact that *G. haemorrhoidalis* larvae were found attached to the pyloric mucosa and duodenum, which are the same sites of localization of *G. nasalis* larvae, in a horse where the latter were not present. Thus, we could speculate that *G. haemorrhoidalis* larvae can survive in horses when *G. nasalis* larvae are absent. This is also corroborated by the fact that, although different species were found in the same horse, larvae of different species were never found mixed together. There was no evidence of any association between age or sex of the host and the prevalence of *Gasterophilus* spp. larvae. Similar observations were recorded by Otranto et al. [23], Pilo et al. [27] in Italy, and Niedźwiedź et al. [22] in Poland. However, Ibrayev et al. [15] and Pandey et al. [24] found in Kazakhstan and Morocco respectively that older animals have fewer larvae than younger ones. They explained this observation by the development of protective immunity resulting from a previous infection. In contrast, Miguélez et al. [20] in their study carried out by ELISA in Spain on exposure of horses to *Gasterophilus* spp., pointed out that older animals were more prone to infection; they suggested thus a cumulative effect of infestation throughout the animal's life. In the same way as age and sex, our study did not show any effect of the breed on the prevalence of *Gasterophilus* spp. In contrast, Liu et al. [18] in China, found that the prevalence of *Gasterophilus* infection depends on the host species. They reported that infection in Przewalski's horses (*Equus przewalskii*) was higher than in the local domestic horse (*E. caballus*) or in Mongolian wild ass (*Equus hemionus*). They explained this observation by the inherent differences in the susceptibility of the three host species. Furthermore, Miguélez et al. [20], reported that the Arabian thoroughbred was found with a higher percentage of antibodies against *Gasterophilus* spp., whereas autochthonous *Pura Raza Gallega* (Galician) horses achieved lower values. They explained their results by the agility gregarious of autochthonous specimens, which could avoid oviposition and therefore their infestation by botfly. It must be mentioned that all horse breeds belong to the same species *E. caballus*.

Regarding the seasonality of the infection by different species of *Gasterophilus*, it is well known that the development from the egg to L2 requires approximately six weeks, while L3 develop for about 8 to 10 months in the gastrointestinal tract of the host [32]. In the present study, the ANOVA analysis revealed that the highest mean number of L3 larvae of *G. intestinalis* was found from January to July ($p = 0.09$). Unlike the L3, no difference was recorded for the L2 ($p = 0.12$). L2 and L3 of *G. nasalis* do not vary through the months ($p = 0.54$ and $p = 0.30$) respectively. No differences for larvae were also found for others species.

No L2 of *G. intestinalis* were present in the stomach from April to July in the first year. The new generation of L2 appears in September, increases gradually to reach a peak in December, and then decreases gradually until March of the second year. A minimum of 6 weeks is required from the time an egg is laid on the hair until it emerges as an L2 in the stomach. This implies that the L2 recovered during September would be the product of the first eggs of the season laid by flies. The high number of L2 of *G. intestinalis* in the stomach of horses during December would be due to the eggs laid in autumn. Usually, it takes 3–5 weeks for the L2 to become L3 in the stomach of horses [23]. Therefore, it seems normal that the L3 will be present in the stomach in large numbers in the 8 months from December of the first year to July of the second year. The decrease of L3 in the stomach of horses in September to November can be explained by their elimination in the feces as they become ready to pupate. These L3 which develop into adult flies between October and December are the main source of infection during this period. Sukhapesna et al. [34] have experimentally demonstrated that higher temperatures hasten embryogenesis and hatching of *G. intestinalis* eggs but reduce their viability. In the studied region, the temperature rises during spring and reaches the highest levels during the months of July and August. The eggs laid in summer can hatch within 2 weeks and probably will not be viable for more than a few weeks, whereas the eggs laid during autumn, when the temperature starts falling, will take longer to hatch and may remain viable for 2–3 months. Therefore, these autumn-laid eggs are probably those infecting horses. However, it seems that after January, the eggs, if they are present on hairs in small number, are not viable and the infection does not occur from February to June. The L2 of *G. nasalis* appear around the same time as *G. intestinalis* (in September of the first year of study). They increase in number to reach a peak a month later in October, declining thereafter to a negligible number in May of the second year. The L2 become L3 in 3–5 weeks. This means that the first new L3 are identified in October and their numbers continue to increase until December. L3 are passed in feces in significant numbers in February and March and the adult flies are, therefore, active from March to October with the peak fly density in summer. No L2 of *G. pecorum* were found during the study. The L3 larvae appear during the rainy months (November–February) of the eastern part of Algeria indicating that the availability

of water plays an important role in its development. A previous study showed that the oviposition sites of *G. pecorum* are often near a water source [17]; only the *Gasterophilus* spp. species lays its eggs on grass [11,28]. Several authors attributed the reduction of the prevalence of gasterophilosis to the extensive use of antiparasitic drugs with larvicidal activity, such as ivermectin and moxidectin. Therefore, the high prevalence of gasterophilosis found in our study would suggest that the treatment of this parasitosis is not extensive in the region, or not correctly applied. This needs to be further elucidated. The frequency per year of the treatments against *Gasterophilus* spp. depends on the biologic cycle. Our results suggest that for horses bred in this region at least, the treatment should be administered in October, corresponding to the peak of *G. nasalis* larvae in the digestive tract. A second treatment may be administered in December, corresponding to the second peak of *G. intestinalis* larvae in the digestive tract.

Conclusion

In the study region, under a semi-arid Mediterranean climate, gasterophilosis is confirmed to be an important parasitosis in horses. Monthly variations of *Gasterophilus* species are distinguished by a marked seasonality of L2 in September for the two dominant parasites *G. intestinalis* and *G. nasalis*, with a first peak in October for *G. nasalis*, and a second peak in December for *G. intestinalis*. The high prevalence of infection found in this study suggests that anthelmintic drugs are not extensively or correctly used. Thus, to control this gasterophilosis, two interventions with a macrocyclic lactone, recommended because of its remanence could be used during this critical period (autumn–winter), and could also act against strongyles found prevalent at this period [4]. Finally, it is advisable to enhance the awareness of horse farmers about the menace of this parasitosis.

Acknowledgments We thank B. Mihi for his revision of English language.

Conflict of interest: The authors do not have any conflict of interest to declare.

References

1. Agneessens J, Engelen S, Debever P, Vercruysse J (1998) *Gasterophilus intestinalis* infections in horses in Belgium. Vet Parasitol 77:199–204
2. Anderson JR (2006) Oestrid myiasis of humans. In: Colwell DD, Hall MJR, Scholl PJ (Eds.), The Oestrid flies: Biology, host–parasite relationships, impact and management. CAB International, Oxford, pp 201
3. Bauer C (1986) Infestation with stomach parasites in horses in North Germany. Dtsch Tierarztl Wochenschr 93:388–9 [Article en allemand]
4. Bentounsi B, Maatallah F (2008) Variations saisonnières de l'excrétion des œufs de strongles par les chevaux en zone subhumide d'Algérie. Rev Elev Med Vet Pays Trop 60:77–9. doi: 10.19182/remvt.10002
5. Cavallero S, Pombi M, Perrone V, et al (2017) *Gasterophilus intestinalis* (Diptera: Oestridae) in the diaphragmatic muscle: an unusual finding. Vet Parasitol 237:117–21. doi: 10.1016/j.vetpar.2017.02.030
6. Côte M (1998) Les régions bioclimatiques de l'est algérien. Revue Rhumel, Université de Constantine (Algérie) 6:57–69
7. Doyle GM, John EH, Craig RR (2003) Control of internal parasites of the horse. University of Tennessee, Institute of Agriculture. 4002:1–8
8. Edwards GT (1982) The prevalence of *Gasterophilus intestinalis* in horses in northern England and Wales. Vet Parasitol 11:215–222
9. Gao DZ, Liu GH, Song HQ, et al (2016) The complete mitochondrial genome of *Gasterophilus intestinalis*, the first representative of the family Gasterophilidae. Parasitol Res 115:2573–9. doi: 10.1007/s00436-016-5002-9
10. Getachew AM, Innocent G, Trawford AF, et al (2012) *Gasterophilus*: a major cause of rectal prolapse in working donkeys in Ethiopia. Trop Anim Health Prod 44:757–62. doi: 10.1007/s11250-011-9961-7
11. Gunn A, Pitt SJ (2012) Parasitology: an integrated approach, p. 431. Wiley-Blackwell, UK, p. 456
12. Höglund J, Ljungström BL, Nilsson O, et al (1997) Occurrence of *Gasterophilus intestinalis* and some parasitic nematodes of horses in Sweden. Acta Vet Scand 38:157–65
13. Huang H, Zhang B, Chu H, et al (2016) *Gasterophilus* (Diptera, Gasterophilidae) infestation of equids in the Kalamaili Nature Reserve, China. Parasite 23:36. doi: 10.1051/parasite/2016036
14. James MT (1947) The flies that cause myiasis in man. U.S. Government Printing Office, Washington, DC, 106–112, USDA Miscellaneous Publication no. 631, p. 182
15. Ibrayev B, Lider L, Bauer C (2015) *Gasterophilus* spp. infections in horses from northern and central Kazakhstan. Vet Parasitol 207:94–8. doi: 10.1016/j.vetpar.2014.11.015
16. Kettle PR (1974) The genus *Gasterophilus* in the horse in New Zealand. N Z Vet J 22:43–5. 10.1080/00480169.1974.34130
17. Liu SH, Hu DF, Li K (2015) Oviposition site selection by *Gasterophilus pecorum* (Diptera: Gasterophilidae) in its habitat in Kalamaili Nature Reserve, Xinjiang, China. Parasite 22:34. doi: 10.1051/parasite/2015034
18. Liu SH, Li K, Hu DF (2016) The incidence and species composition of *Gasterophilus* (Diptera, Gasterophilidae) causing equine myiasis in northern Xinjiang, China. Vet Parasitol 217:36–8. doi: 10.1016/j.vetpar.2015.12.028
19. Mashayekhi M, Ashtari B (2013) Study of Gasterophilus role in equine gastric ulcer syndrome in Tabriz area. Bull Env Pharmacol Life Sci 2:69–72
20. Miguélez S, Araújo AM, Francisco I, et al (2016) Exposure to *Gasterophilus* spp. in horses in NW Spain by ELISA. J Entomol Zool Stud 4:621–4
21. Mukbel R, Torgerson PR, Abo-Shehada M (2001) Seasonal variations in the abundance of *Gasterophilus* spp. larvae in donkeys in northern Jordan. Trop Anim Health Prod 33:501–9
22. Niedźwiedź A, Borowicz H, Nicpoń JM (2013) Prevalence study in horses infected by *Gasterophilus* sp. in an eastern region of Poland. Vet Parasitol 191:94–6. doi: 10.1016/j.vetpar.2012.08.023

23. Otranto D, Milillo P, Capelli G, Colwell DD (2005) Species composition of *Gasterophilus* spp. (Diptera, Oestridae) causing equine gastric myiasis in southern Italy: Parasite biodiversity and risks for extinction. Vet Parasitol 133:111–8
24. Pandey VS, Ouhelli H, Elkhalfane A (1980) Observations on the epizootiology of *Gasterophilus intestinalis* and *G. nasalis* in horses in Morocco. Vet Parasitol 7:347–56. doi: 10.1016/0304-4017(80)90055-2
25. Pandey VS, Ouhelli H, Verhulst A (1992) Epidemiological observations on *Gasterophilus intestinalis* and *G. nasalis* in donkeys from Morocco. Vet Parasitol 41:285–92. doi. 10.1016/0304-4017(92)90087-P
26. Panitz E (1978) Occurrence of second and third instars of *Gasterophilus intestinalis* and *G. nasalis* in horses in the Mid-Atlantic United States. Vet Parasitol 4:161–6. doi. 10.1016/0304-4017(82)90044-9
27. Pilo C, Altea A, Scala A (2015) Gasterophilosis in horses in Sardinia (Italy): effect of meteorological variables on adult egg-laying activity and presence of larvae in the digestive tract, and update of species. Parasitol Res 114:1693–702. doi: 10.1007/s00436-015-4352-z
28. Principato M (1989) Observations on the occurrence of five species of *Gasterophilus* larvae in free-ranging horses in Umbria, central Italy. Vet Parasitol 3:173–7. doi: 10.1016/0304-4017(92)90087-P
29. Rehbein S, Visser M, Winter R (2013) Prevalence, intensity and seasonality of gastrointestinal parasites in abattoir horses in Germany. Parasitol Res 112:407–13. doi: 10.1007/s00436-012-3150-0
30. Royce LA, Rossignol PA, Kubitz ML, Burton FR (1999) Recovery of a second instar Gasterophilus larva in a human infant: a case report. Am J Trop Med Hyg 60:403–4
31. Slivinskav K, Kharchenko V, Wróblewski Z, et al (2016) Parasitological survey of Polish primitive horses (*Equus caballus gmelini* Ant.): influence of age, sex and management strategies on the parasite community. Helminthologica 53:233–42
32. Soulsby EJL (1968) Helminths, arthropods and protozoa of domesticated animals, 6th ed. Baillière, Tindall & Cassell, London, UK, p. 824
33. Studziňska MB, Wojcieszak K (2009) *Gasterophilus* spp. botfly larvae in horses from the south-eastern part of Poland. Bull. Vet Inst Pulawy 53:651–5
34. Sukhapesna V, Knapp FW, Lyons ET, Drudge JH (1975) Effect of temperature on embryonation, development and egg hatchability of the horse bot, *Gasterophilus intestinalis* (Diptera: Gasterophilidae). J Med Entomol 12:391–2
35. Sweeney HJ (1990) The prevalence and pathogenicity of *Gasterophilus intestinalis* larvae in horses in Ireland. Ir Vet J 43:67–73
36. Yang JY, Zhang D, Hu DF, et al (2013) The injury caused by myiasis of *Gasterophilus* in horse. Zhongguo Xu Mu Shou Yi 40:177–80
37. Zumpt F (1965) Myiasis in man and animals in the Old World: a textbook for physicians, veterinarians and zoologists. Butterworths, London, p. 267