



***Gasterophilus spp.* larvae infection rates and seasonal variation in donkeys autopsied in Nyala, South Darfur, Sudan**

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ABSTRACT

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During a twelve months survey, 92 donkeys were necropsied for *Gasterophilus spp.* larvae in Nyala, South Darfur, Sudan. In the course of the survey, *Gasterophilus intestinalis* were recorded from 92.4% of examined donkeys whereas 77.2% of animals were infected with *Gasterophilus nasalis*. The percentage of infected animals with *Gasterophilus haemorrhoidalis* was 72.2 %. The overall infection rate with the parasites was 92.4%. The infection rate of *Gasterophilus* larvae was high throughout the year; it was higher in the cold dry season followed by hot wet and hot dry seasons. Most of infection was mixed, 60(70.6%) of the infected donkeys showed triple infection, while 21(24.7%) harboured double infection and only 4 (4.7%) of examined donkeys had single infection. The prevalence rate was differed between age groups, showing high percentage in donkeys more than five years old than other age groups of animals. Middle-aged animals gave higher average number of larvae per animal infected than other age groups. With the flowing reports of adverse health effects of *Gasterophilus* larvae, these findings provide a base for further studies to determine the infection rate in donkeys from other regions of the Sudan as well as evaluation of control measurements.

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## INTROOUCTION

The donkey (*Equus asinus L.*) is one of the most important domestic animals, contributing to many social and economic sectors of the resource-limited communities (Svendsen, 1997). Despite the large number of donkeys, and the valuable services they provide in Sudan, the attention given to the health and welfare of these animals is minimal. Investigating the different health and welfare problems is crucial to improve the health and productivity of and for the better utilization of these animals (Getachew *et al.*, 2012).

*Gasterophilus* larvae (Diptera: *Oestridae*) are common obligate parasites in the gastrointestinal tract of equine (including horses, donkeys, and Zebras), and cause gastrointestinal myiasis. Eight species were reported to occur in equids (Zumpt, 1965). The 2<sup>nd</sup> - and 3rd stage larvae are normally found attached to the mucosa of the stomach (typically, *G. intestinalis*), duodenum (*G. nasalis*, *G. haemorrhoidalis*), or rectum (*G. haemorrhoidalis*, *G. inermis*), where they cause focal superficial mucosal ulceration (Principato, 1988). The data pertaining to significant adverse health effects of gasterophilus infection were increasing, such as reports of gastric perforation and secondary peritonitis (Waddell, 1972, Dart *et al.*, 1987).

The available literature reveals that there is valuable information on larvae of *Gasterophilus spp.* infesting horses and ponies (Zumpt, 1965; Drudge *et al.*, 1975; Hatch *et al.*, 1976; Pantiz, 1978; Pandey *et al.*, 1980; Sequeira *et al.*, 2001; and Domenico *et al.*, 2005). To our knowledge, no recent data were available concerning the incidence of *Gasterophilus spp.* infecting donkeys (Hilali *et al.*, 1987; Pandey and Eysker, 1988; Pandey *et al.*, 1992 and Mukbel *et al.*, 2001; and Seri *et al.*, 2004).

The veterinary importance of gasterophilosis has largely dealt with the damage the larvae

produce in the gastric and duodenal mucosa. Although the current concept is that they are considered to be well tolerated by their hosts, they have been incriminated in inducing gastric erosion, ulcers, gingivitis, glossitis, subserosal abscesses and peritonitis in horses (Coles and Pearson, 2000, Sequeira *et al.*, 2001, Lapointe *et al.*, 2003, Smith *et al.*, 2005, Gokcen *et al.*, 2008, Studzinska and Wojcieszak, 2009). Getachew *et al.*, (2012), reported that 83.6% of rectal prolapse cases were associated with *Gasteriphilus nasalis*. Data obtained from study conducted in Tabriz (Iran) showed that out of 16 horse cases which were referred to be suspected to gastric ulcer (diagnosis based on clinical signs), 5 cases were suffered from *Gasterophilus* which caused gastric ulcer. Those animals were died because of cachexia, in necropsy 3 cases showed the larva in the gastric mucosa (Mashayekhi and Ashtari, 2013).

The presence of *Gasterophilus* larvae in the stomach and the other parts of gastrointestinal tract of equines has been associated with many pathological lesions. Gastro and intestinal ulceration, gut obstructions or volvulus, rectal prolapsed, anaemia, diarrhea and digestive disorders were reported (Pandey *et al.*, 1980; Principato, 1988; Cogley and Cogley, 1999; Sandin *et al.*, 1999; Sequeira *et al.*, 2001; and Otranto *et al.*, 2005). Perforation or rupture of the gastrointestinal tract with resulting peritonitis has been documented as a sequel of *Gasterophilus* infection (van der Kolk *et al.*, 1989).

Although botfly larvae are known to attach to aberrant sites within the digestive tract, Lapointe and his colleagues (2003) reported the first case of deep penetration of the colon by a gasterophilus larva, with the resulting leakage of intestinal content leading to septic peritonitis.

They also have some zoonotic potential as they are occasionally reported to affect humans, where they are found subcutaneously or in the digestive tract (Zumpt 1965, Royce *et al.*, 1999, Anderson 2006). In addition, subcutaneous-creeping or ophthalmo-myiasis by *Gasterophilus spp.* first and second stage larvae (L1, L2) have been reported by Currier *et al.*, (1995) and Royce *et al.*, (1999).

In Sudan, to our knowledge there was no detailed study to report on the infection rate and different species of *Gasterophilus spp.* larvae in donkeys. Hence, the present study reports on the infection rate with *Gasterophilus spp.* and their seasonal variation in donkeys from Nyala, Sudan.

## MATERIALS and METHODS

*Site of study.* Nyala town, located in South Darfur State was selected to conduct this study. The climate is characterized by a hot wet season (July-October), cold dry season (November-February) and a dry hot season (March-June).

*Experimental animals.* 92 donkeys of both sexes were used in this study. They were purchased from a local livestock market in Nyala. They were chosen from animals grazed on natural land pasture throughout the year. The donkeys were acquired at different periods of the year (5-13 animals every month). The interval between the acquisition of animals and necropsy examination ranges between 3-7 days. The study period extended from May, 2005 to April, 2006. Donkeys were sacrificed for postmortem examination by carotid artery bleeding. The animals were starved for 2 days before necropsy. The stomach, small intestine, caecum, ventral, dorsal colon and rectum were separated by a double ligature and were then cut from each other. Each part was opened as soon as possible; the *Gasterophilus* larvae were collected and

differentiated into species according to Zumpt, (1965).

*Statistical analysis.* Data were summarized in terms of percentage and mean±SD to describe infection rates and seasonal variation. Software used was SPSS for windows, version 14.0.

## RESULTS

The overall prevalence of infection with *Gasterophilus spp.* larvae was 92.4% (85 out of 92) as shown in table (1). A total of 13438 of larvae were collected with a mean intensity of 185.0 larvae per infected animal. Larvae were collected throughout the observation period with the highest total number collected in August (2984) while the lowest mean burden was registered in December (475) (Table, 1).

The prevalence rate, the total and mean number of larvae collected are depicted in Tables (2, 3 and 4) for *G. intestinalis*, *G. nasalis* and *G. haemorrhoidalis*, respectively. *Gasterophilus intestinalis* was the most common species (92.4%) followed by *Gasterophilus nasalis* (77.2%) and *Gasterophilus haemorrhoidalis* (72.2%). The effect of the age of examined animals on the prevalence rate and total larvae counted from infected donkeys is shown in table (5). The prevalence rate differed between age groups. The prevalence rate is very high in donkeys more than five years old than other age groups of animals. Middle-aged animals gave higher average number of larvae per animal infected than other age groups.

The effect of season on the larval burden was shown in table (6). The nature of infection with *Gasterophilus* larvae of infected donkeys was mixed. The majority of the animals were infected by three species (70.6%) or two species (24.7%) and only 4.7% of infested animals harboured single type of the parasite. The maximum number of larvae recovered in the same donkey was noted in August when 1054 were collected

from a single animal (i.e., 402 *G. intestinalis* and 143 *G. nasalis* in the stomach respectively and 509 *G. haemorrhoidalis* in the rectum).

Ulcers and erosions were found in infected animals in each part where the parasite found attached to the mucosa. *Gasterophilus intestinalis* larvae were recovered from the oesophageal region of the stomach, while *Gasterophilus nasalis* were recovered from the pyloric part of the stomach and the first part of the duodenum. *Gasterophilus haemorrhoidalis* were recovered from the rectum.

## DISCUSSION

The present study showed that *Gasterophilus intestinalis* prevalence (92.4%) was more dominant than *Gasterophilus nasalis* (77.2%) and *Gasterophilus haemorrhoidalis* (72.2%). This prevalence rate is high when compared to the work of Botros (1942) who found that (60%) of donkeys and horses were infected with *Gasterophilus intestinalis* and (40%) with *Gasterophilus nasalis*, similarly, Lyons *et al.*, (2000) found a very low infection rate by bots of *Gasterophilus intestinalis* (14%) and *Gasterophilus nasalis* (2%) in Kentucky. The infection rates with bots reported here were in accordance with the work of Hilali and his colleagues (1987) who reported (98.3%) and (87.3%) for *Gasterophilus intestinalis* and *Gasterophilus nasalis*, respectively. The same result is strongly supported by Mukbel *et al.*, (2001) who reported the occurrence of *Gasterophilus intestinalis* (93%) among donkeys in Jordan. However, the infection rate of *Gasterophilus nasalis* (48%) is a much lower than that reported in this survey. The current survey showed that high infection by *Gasterophilus haemorrhoidalis* (72.2%) was also evident; postmortem examination revealed five positive cases with rectal prolapse and under careful examination clusters of *Gasterophilus*

*haemorrhoidalis* larvae were found attached to the wall of the rectum which may be due to the irritation of the rectal wall. This finding is supported by works of Yilma *et al.*, (1991) who reported that rectal prolapse is quite common in donkeys of Ethiopia which is associated with infection by *Gasterophilus larvae*.

As the second and third instar larvae inhabit the gastrointestinal tract and attach to the stomach and intestine, multiple complications may arise. Larvae present in large numbers in the stomach can cause blockages and lead to colic. Large numbers of larvae impact the host by damaging the tissue of the stomach or the gut lining and consuming the nutrients that would otherwise be beneficial to the hosts' well-being. Other health issues that may develop due to a severe infestation of these larvae include: chronic gastritis, ulcerated stomach, esophageal paralysis, peritonitis, stomach rupture, squamous cell tumors, and anemia (Brocard and Pfister, 1991; Høglund *et al.*, 1997).

In other studies conducted in various regions of the world, prevalence of infestation ranged from 9% to 100% including 9% in Germany (Ribbeck *et al.*, 1983), 12.3% in Sweden (Høglund *et al.*, 1997), 43% in Ireland (Sweeney, 1990), 53% in England and Wales (Edwards, 1982), 58% in Belgium (Agneessens *et al.*, 1998), 65% in Switzerland (Brocard and Pfister, 1991), 82.2% in Italy (Otranto *et al.*, 1975), 100% in Morocco (Pandey *et al.*, 1980).

There is no doubt that extensiveness of *Gasterophilus* spp. infection is reported in warmer countries (Drudge *et al.*, 1975). The number of botfly larvae in donkeys seems interesting. Our examinations revealed that the intensity of infection in donkeys from Nyala was high, 185 larvae per infected donkey.

There is no evidence for a significant relationship between prevalence and mean intensity with age and sex of the host

(Agneessens *et al.*, 1998, Mukbel *et al.*, 2001; Gokcen *et al.*, 2008). But the higher prevalence in female than male animals was noted by another author (Ica and Yildirium, 2005). Moreover, Edwards (1982) reported that prevalence of infection and mean larval burdens declined with increasing age of host.

## CONCLUSION

To decide about state of gastrophilosis in donkeys in this region, infestation in the 85 of the 92 donkeys autopsied give strong indication of the current situation of gastrophilosis in Darfur region. Several risk factors may associate this percentage viz: absence of treatment with anthelmintics, bad management factors such as poor maintenance conditions and animals work under severe conditions, free grazing animals, which expose them to contact with female botflies, to live with other equine reservoir to these larvae causing gastrointestinal myiasis.

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Table 1: Monthly prevalence of *Gasterophilus* larvae of donkeys found to be positive during the survey period

Month	No. examined	No. positive (%)	Total No. of larvae
January	6	6 (100%)	666
February	7	7 (100%)	843
March	6	6 (100%)	731
April	7	7 (100%)	664
May	11	9 (81.8%)	2091
June	13	11 (84.6%)	1136
July	6	5 (83.3%)	709
August	7	7 (100%)	2984
September	5	5 (100%)	600
October	10	10 (100%)	1838
November	8	7 (87%)	701
December	6	5 (83.3%)	475
Total	92	85 (92.4%)	13438

Table 2: Prevalence rate and Mean ( $\pm$  SD) monthly number of larvae of *Gasterophilus intestinalis* recovered from donkeys in Nyala during the survey period.

Month	No. of Animals examined	No. of Animals infected (%)	Total no. of larvae counted	Mean $\pm$ SD No. of larvae counted
May, 2005	11	9(81.8)	1315	146.11 $\pm$ 172.59
June, 2005	13	11(84.8)	598	54.36 $\pm$ 49.94
July,2005	6	5 (83.3)	402	80.00 $\pm$ 48.62
August,2005	7	7 (100)	1369	190.43 $\pm$ 121
Sept., 2005	5	5 (100)	170	34.20 $\pm$ 18.18
October,2005	10	10 (100)	1317	137.46 $\pm$ 101.52
November,2005	8	7 (87.5)	567	81.00 $\pm$ 60.22
December,2005	6	5 (83.3)	277	55.40 $\pm$ 28.50
January, 2006	6	6 (100)	461	76.83 $\pm$ 44.45
February,2006	7	7 (100)	599	85.57 $\pm$ 5.65
March ,2006	6	6 (100)	443	73.83 $\pm$ 9.73
April ,2006	7	7 (100)	469	62.71 $\pm$ 71
Overall	92	85 (92.4)	7987	
Larvae/animal infected				93.96

Table 3: Prevalence rate and Mean ( $\pm$  SD) monthly number of larvae of *Gasterophilus nasalis* recovered from donkeys in Nyala during the survey period.

Month	No. of Animals examined	No. of Animal, infected (%)	Total no. of larvae counted	Mean $\pm$ SD No. of larvae counted
January, 2006	6	6 (100)	181	21.83 $\pm$ 2.83
February,2006	7	6 (85.7)	198	33.00 $\pm$ 4.06
March ,2006	6	6 (100)	231	38.50 $\pm$ 3.03
April ,2006	7	7 (100)	132	11 $\pm$ 18.86
May, 2005	11	8 (72)	466	58.38 $\pm$ 51.33
June, 2005	13	8 (61.5)	157	19.63 $\pm$ 18.28
July,2005	6	5 (83.3)	114	22.80 $\pm$ 16.38
August,2005	7	7 (100)	636	90.86 $\pm$ 59.38
Sept., 2005	5	5 (100)	317	62.20 $\pm$ 49.43
October,2005	10	9 (100)	413	32.88 $\pm$ 28.56
November,2005	8	2 (25)	11	5.5 $\pm$ 2.12
December,2005	6	2 (33.3)	139	69.50 $\pm$ 44.92
Overall	92	71 (77.2)	2995	
larvae/animal infected				32.01

Table 4: Prevalence rate and Mean ( $\pm$  SD) monthly number of larvae of *Gasterophilus haemorrhoidalis* recovered from donkeys in Nyala during the survey period.

Month	No. of Animals examined	No. of Animals infected (%)	Total no. of larvae counted	Mean No. ( $\pm$ SD) of larvae counted
January, 2006	6	6 (100)	74	12.33 $\pm$ 3.88
February, 2006	7	5 (71.4)	46	9.20 $\pm$ 8.62
March , 2006	6	4 (66.7)	37	14.25 $\pm$ 7.93
April , 2006	7	6 (85.7)	63	12.00 $\pm$ 11.60
May, 2005	11	8 (72.7)	276	34.50 $\pm$ 34.56
June, 2005	13	10 (76.9)	381	37.20 $\pm$ 34.96
July, 2005	6	4 (66.7)	193	48.25 $\pm$ 25.20
August, 2005	7	7 (100)	979	139.86 $\pm$ 173.45
Sept., 2005	5	5 (100)	113	22.60 $\pm$ 17.50
October, 2005	10	6 (60)	108	20.20 $\pm$ 13.75
November, 2005	8	4 (50)	123	29.25 $\pm$ 26.35
December, 2005	6	5 (83.3)	59	11.80 $\pm$ 11.99
Overall	92	70 (76.1)	2472	206.00
Larvae/animal infected				26.87

Table 5: Prevalence rate of *Gastrophilus* larvae counted from infected animals from different age groups.

Age group (years)	No .of animal examined	No. of animal infected	Prevalence rate (%)	Total larvae counted	average no. of larvae /animal infected
≤ 1.5	9	7	7.61	466	66.57
1.5-3.5	25	23	25	2329	101.26
3.5-5.5	20	18	19.57	3808	211.55
≥ 5.5	38	37	40.22	5988	161.83

Table 6: Seasonal mean *Gasterophilus* larvae  $\pm$ SD recovered from stomach and rectum of infected donkeys during May 2005-April 2006.

Seasons	<i>Gastrophilus intestinalis</i>	<i>Gastrophilus nasalis</i>	<i>Gastrophilus haemorrhoidalis</i>
Dry cold	76.2 $\pm$ 9.2	29.9 $\pm$ 7.9	14.8 $\pm$ 3.3
Hot dry	84.7 $\pm$ 17.5	34 $\pm$ 6.8	28.3 $\pm$ 5.7
Hot wet	121.5 $\pm$ 19.8	53 $\pm$ 9.7	66 $\pm$ 24.1