

## Dynamics of infestation by *Rhinoestrus usbekistanicus* in donkeys (*Equus asinus*) in Tunisia

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### Summary

Examination of a total of 125 slaughtered donkeys in Tunisian slaughterhouses revealed the presence of 1,508 larvae of *Rhinoestrus usbekistanicus* in their nasal cavities, with an overall infestation prevalence of 52.8%. The highest overall intensity (number of larvae per infested donkey) and abundance (number of larvae per donkey examined) of infestation were reported in winter (35.6 and 12.1, respectively). Larvae 1 (L1) were the most frequent stage and reach a peak prevalence of 100% during November. The number of larvae 2 (L2) and 3 (L3) was low and the maximum was recorded during March and April. Post-mortem observations showed that infestation intensity increases with age. The ethmoid bone was the most frequently infected portion of the nasal cavity (83.75%). This is the first study on nasal bots in donkeys in Tunisia.

### Keywords

Donkey – Nasal bot – Nasal cavity – *Rhinoestrus usbekistanicus* – Tunisia.

## Introduction

The total number of horses, donkeys and mules in Tunisia in 2017 was estimated to be 57,254, 241,646 and 82,649, respectively. These animals are distributed particularly in the centre of Tunisia (51.7%) (1).

Nasal myiasis of equidae are caused by larvae of flies belonging to the family Oestridae and the genus *Rhinoestrus*. Only *Rhinoestrus purpureus* and *Rhinoestrus usbekistanicus* parasitise horses, donkeys and zebras (2). In Africa, these species are known to be present in sub-Saharan regions (3, 4).

In equids, nasal myiasis is characterised by irritation of the nasal cavities, sinuses and pharynx. This infestation can also cause lesions of the upper respiratory tract and even the lungs, with mild exudative inflammation, emphysema, interstitial pneumonia and eosinophilic infiltration (5). Importantly, *Rhinoestrus* spp. may also cause ophthalmomyiasis and conjunctivitis in humans (6). Outbreaks of nasal myiasis caused by *Rhinoestrus* spp. have been registered in Russia, with a prevalence of 11.2% in horses from 27 farms and a mortality rate of 82% (7). This parasite has been detected in donkeys in Egypt (3), Senegal (4, 5) and Niger (8). Myiasis caused by *Rhinoestrus* larvae was thought to be confined to Asiatic and African countries, but it has been reported in Europe, specifically in southern Italy with a prevalence of up to 6% (9). However, it has never been reported in Algeria and Libya. In Morocco, the presence of this parasite was observed by Dakkak, Khallaayoune and Dorchies (unpublished data) in the area of Rabat in seven animals among nine slaughtered donkeys and mules. The aim of this survey was to study the presence and infestation dynamics of *Rhinoestrus* spp. infesting donkeys in Tunisia.

## Materials and methods

The survey was carried out monthly during one year (from October 2013 to September 2014). A total of 125 slaughtered donkeys, belonging to the local breed, from the north west (governorates of

Jendouba, Kef and Siliana) and the centre of Tunisia (governorates of Mahdia, Kairouan and Kasserine) were examined. The donkeys' ages were estimated by examining the incisors of each animal. The head was incised longitudinally and different regions of the nasal cavity were examined. Larvae were first identified macroscopically and collected from the nasopharyngeal cavity. Mucus was collected in a petri dish and examined under a stereomicroscope. Each compartment of the nasal cavity was rinsed with isotonic sodium chloride solution (0.9%) in a container and the solution was sieved to look for the presence of larvae. Larvae were identified under a stereomicroscope according to the key by Zumpt (2) and the observations of Guitton *et al.* (10) (Table I).

**Table I****Criteria for definitive identification of larval instars of *Rhinoestrus usbekistanicus* (10)**

Larval instar	Pseudocephalon	Segments
First larval instar (L1)	<p>Antennary lobes: very prominent, separated by a deep groove</p> <p>Buccal funnel: strongly structured</p> <p>Anterior hooks: large, directed ventrally and obliquely towards the exterior</p> <p>Spine-crown: almost completely surrounds the anterior end with a few rows of spines</p>	<p><b>Segments 3 to 11</b></p> <p>Ventrally: Ventral spines arranged regularly along the body in four complete rows on the anterior part of each metamere. Sometimes, some additional spines are present in an incomplete row</p> <p>Dorsally: Rows of spines are interrupted medially from the fourth segment. On the sixth segment only, a few spines are present laterally</p> <p><b>Segment 12</b></p> <p>Ventrally: There are six to seven rows of spines</p> <p>Dorsally: Many small spines present on a few rows medially. Stigmatic plates are large, having few pores which are very big. Anal outline is surrounded by numerous, dumpy, long and sharply pointed spines. Stigmatic margins show ten sensorial ciliated papillae which are regularly distributed</p>
Second larval instar (L2)	<p>Antennary lobes: more prominent than in the first larval instar with triangular shape dorsally</p> <p>Buccal funnel: structured and the spine-crown is reduced in the number of rows and in the diameter of spines</p> <p>Cephalic hooks: reduced in length, directed less obliquely than for the first instar. The second segment carries medio-ventral hair-like structures which are probably sensorial</p>	<p><b>Segments 3 to 11</b></p> <p>Ventrally: Three to four rows of spines regularly found along the whole body on anterior end of each metamere. Spines are strong and cone shaped. The third and fourth segments show identical sensorial structures to the second segment. On the other segments they are replaced by an ovalaire structure without an appendage</p> <p>Dorsally: Segments 3 and 4 carry uninterrupted rows of spines. Segment 5 shows a medio-dorsal interruption. The following segments (6 and 7) show a few lateral spines. The last segments (8–11) are bare</p> <p><b>Segment 12</b></p> <p>Stigmatic plates very small and triangular shaped with rounded angles. Pores are big and shaped like coffee-beans. There are no spines on the dorsal margin of the stigmatic plates. Numerous and strong spines lie on both sides of the anal outline. The stigmatic margins showed ten ciliated sensorial papillae</p>

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Third larval instar (L3)	<p>Antennary lobes: less prominent than for the precedent instars</p> <p>Cephalic hooks: long, massive and sharply pointed, directed ventrally and slightly obliquely</p> <p>Spine-crown: uninterrupted and shows many rows of triangular spines</p>	<p><b>Segments 2 to 11</b></p> <p>Ventrally: Massive triangular spines with a wide base and pointed extremity in three or four rows for each metamere. The sensorial structures are the same as those of the second instar</p> <p>Dorsally: Rows of spines are interrupted medially on segments 3, 4 and 5. The 5th segment shows a few spines laterally. Segments 7 to 11 are bare</p> <p><b>Segment 12</b></p> <p>A few spines are present medially on the dorsal face. The internal channel is long. The central button is included in peritremes. The stigmatic margins bear ten sensorial ciliated papillae. Sometimes, near these, are found orifices which are probably excretory papillae. Heaped structures are visible on the ventral margin of the peritremes. Stigmatic plates are of average size but more developed than for the second instar. The pores are very small and very numerous. The anal outline is surrounded by numerous and dumpy spines. The male-front, in its narrowest point, measures about one-third of the eye-length. The parafrotales bears well-separated and almost symmetrical tubercles. The mesonotum is without black or black-brown wheals. All the dorsal face shows tubercles with central spines and wide patches of very small hairs between the tubercles. Ventrally, the thorax is entirely covered with long, fine hairs. Few tubercles are visible on the lateral side of the abdomen</p>
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## Epidemiological indicators

Three epidemiological indicators were estimated, using the following formulas (11):

Prevalence of infestation =  $100 \times \text{number of infested donkeys} / \text{number of donkeys examined}$

Infestation intensity =  $\text{number of larvae collected} / \text{number of infested donkeys}$

Abundance of infestation =  $\text{number of larvae collected} / \text{number of donkeys examined}$

## Statistical analysis

The infestation prevalence and intensity according to age were compared using Epi Info 6 (12). A chi-square Mantel–Haenszel test was performed. Percentages were expressed as the mean  $\pm$  standard deviation (SD) and an effect was considered significant at a level of confidence of 5% or less ( $p \leq 0.05$ ) (13).

## Results

### Parasitological indicators

A total of 1508 *Rhinoestrus usbekistanicus* larvae of different stages were collected from the 125 donkeys examined (Figs 1, 2 and 3). The overall prevalence was 52.8% (66/125) (Table II). The highest prevalence of infestation was observed during autumn (96.7%) and the lowest in summer (15.4%) (Fig. 4). The overall intensity and abundance of infestation were 22.8 and 12.1, respectively (Table II). The highest abundance and intensity of infestation were observed in winter (24 and 35.6, respectively), and the lowest were observed in summer (3.5 and 0.5, respectively) (Fig. 4). There was an increase in infestation intensity from October to December (63.5) and a second peak in February (39.0). There was no significant difference in the prevalence and intensity of infestation of donkeys by *R. usbekistanicus* according to age ( $p > 0.05$ ) (Table II). Larvae 1 (L1)

were the most frequent stage, with a maximum of 255 larvae and a peak prevalence of 100% during November (Fig. 5). Their maximum abundance was 50.8 and the maximum infestation intensity was 63.5, during December. The maximum number of larvae 2 (L2) and 3 (L3) was six per animal during March and April, respectively (Fig. 5).



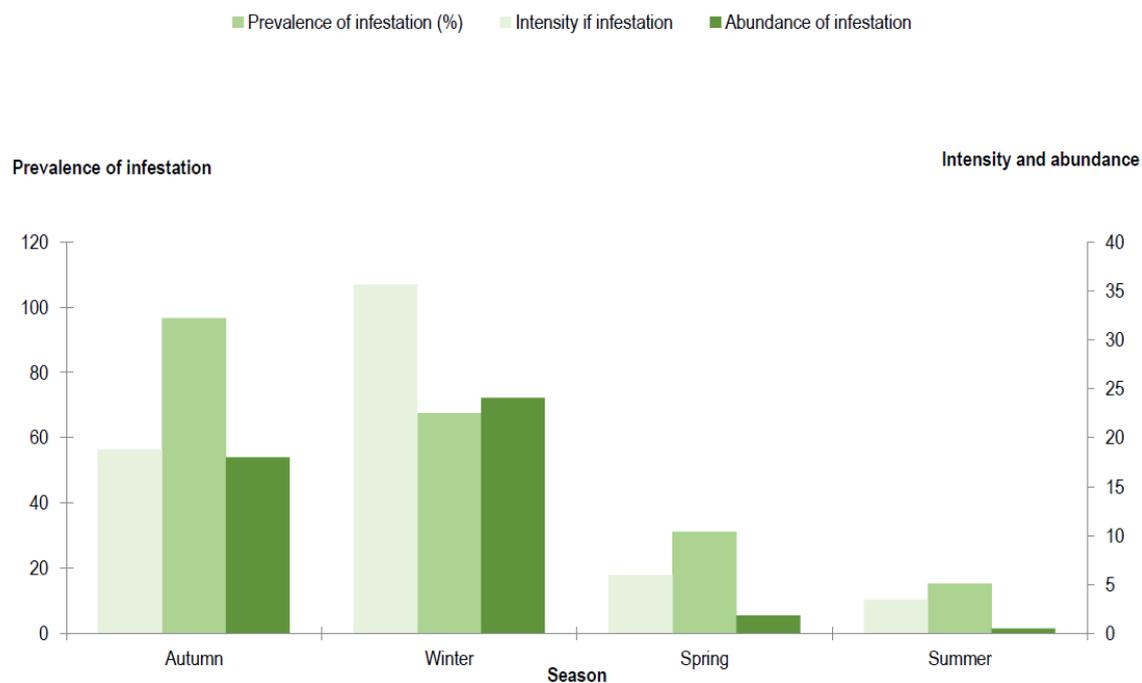
**Fig. 1**  
Stage 1 larva (L1) of *Rhinoestrus usbekistanicus*



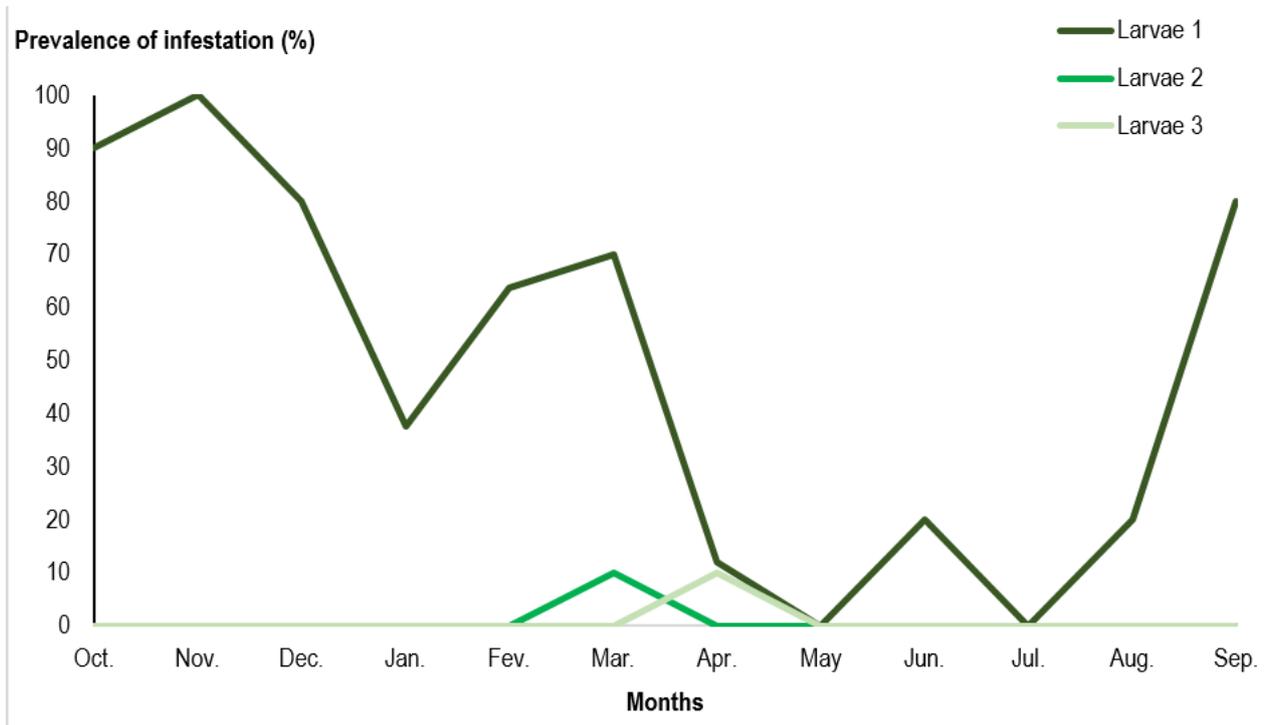
**Fig. 2**  
Stage 2 larva (L2) of *Rhinoestrus usbekistanicus*



**Fig. 3**  
**Stage 3 larva (L3) of *Rhinoestrus usbekistanicus***



**Fig. 4**  
**Seasonal parasitological indicators of infestation of donkeys by *Rhinoestrus usbekistanicus***



**Fig. 5**  
**Monthly infestation prevalence of infestation of donkeys by *Rhinostyrus usbekistanicus* in Tunisia**

**Table II**  
**Parasitological indicators of infestation of donkeys by *Rhinostyrus usbekistanicus* according to age group**

Parasitological indicators	Age group			Total
	≤ 1 year	1-5 years	> 5 years	
Donkeys examined	36	53	36	125
Donkeys infested	25	24	17	66
Number of larvae	293	769	446	1508
Prevalence (% ± SD)	69.4 ± 0.08	45.3 ± 0.07	47.2 ± 0.08	52.8 ± 0.04
Intensity	11.7	32.0	26.2	22.8
Abundance	8.1	14.5	12.4	12.1

SD: Standard deviation

## Lesions

During the examination of the donkeys' heads post-mortem, lesions in the nasal cavity (congestion, pus and nodules) were observed that may have been related to infestation by *R. usbekistanicus* (Table III and Fig. 6). The ethmoid bone was the most frequently infected portion of the nasal cavity (83.75%).



a



b



c

**Fig. 6****Lesions of the nasopharyngeal region**

- a) Dark-coloured dorsal nasal turbinate
- b) Slight congestion of the left dorsal nasal turbinate
- c) Thick and dark-coloured secretion in the right frontal sinus

**Table III****Lesions of the nasopharyngeal region in Tunisian donkeys**

Lesions	Positive/examined (prevalence in % $\pm$ SD)	Number of larvae in donkeys presenting lesions (percentage of larvae, %)
Intense congestion	44/125 (35.2 $\pm$ 0.04)	861 (57.1)
Abundant mucus	36/125 (28.8 $\pm$ 0.04)	400 (26.5)
Tumour	1/125 (0.8 $\pm$ 0.01)	0
Pus	7/125 (5.6 $\pm$ 0.02)	30 (2)
Nodule	3/125 (2.4 $\pm$ 0.01)	15 (1)
Overall	65	1306 (86.6)

SD: Standard deviation

## Discussion

In this study, the donkeys were infested by a single species of nasal bot: *R. usbekistanicus*. It had been thought that this myiasis was restricted to Asiatic and African countries (2, 3, 4, 5, 7, 8, 14), but it was reported for the first time in Europe (in southern Italy) in 2001 (15). In this study, a total of 1508 larvae of *R. usbekistanicus* were found in the nasal cavities of 125 donkeys. This finding differed from what was observed in Europe: in Italy, morphological examination of L3 revealed the presence of 8% *R. purpureus*, 8% *R. usbekistanicus* and 84% of larvae presenting an intermediate morphology (16). In addition, in the Apulia region (south-east Italy) and Sicily, Otranto *et al.* (9) examined 212 horses and 120 horses with 2 donkeys, separately. They showed the presence of specimens with characteristics of both *R. usbekistanicus* and *R. purpureus*. The presence of combined features in the L3 of *Rhinoestrus* collected from the same animals could be explained by the existence of a single species of *Rhinoestrus* which presents different morphotypes, or the presence of two morphotypes (1 and 2) of *Rhinoestrus* spp. (*R. usbekistanicus* like and *R. purpureus* like), perhaps because southern Italy is midway between eastern and African countries (9). *Rhinoestrus usbekistanicus* has also been found in horses and donkeys in Senegal (4, 5) and in Niger (8).

This work revealed a high prevalence of infestation with *R. usbekistanicus* in donkeys (52.8%). This prevalence is similar to that obtained by Abo-Aziza *et al.* (17), who found that 50% (40/80) of donkeys examined were infested with one or more equine nasal bots (*Rhinoestrus* spp.) larvae. However, this prevalence is lower than that found by Hilali *et al.* in Egypt (18). That study revealed that all of the donkeys examined during a one-year period harboured one or more larval stages of *Rhinoestrus* spp. (100%). In Turkey, Tuzer and Tan also reported a prevalence of 100%, in horses (19). Lower prevalence values have been reported in other countries, including Italy: 6.13% and 4.16% in the Apulia and in Sicily regions, respectively (9). Milillo *et al.* (20) found an infestation prevalence of 6.13% in 212 horses examined in Italy. These lower prevalence values may be due to the

treatment of animals with avermectins and/or to differences in the climatic conditions and hence the epidemiological environment. Otranto *et al.* (9) reported no statistically significant difference in the prevalence of infestation according to age ( $p > 0.05$ ). In the survey of Otranto *et al.*, animals were pooled in three age groups and the absence of difference between age groups could have been due to the pooling of the animals (< 1, 1–2 and > 2 years) (9). Climatic factors, soil typology and the immune status of equids play an important role in larval growth and in the cycle of *Rhinoestrus* spp. (21).

In this trial, a peak of infestation (100%) was found in November, followed by a high prevalence in December (80%). The mean prevalence of L1 was 47.8% and the mean prevalence for L1 and L2 was 0.8%. Monthly analysis of L1 showed that 95% of them were present between September and February, L2 were found during March, and L3 during April.

Mula *et al.* (16) analysed the dynamics of *Rhinoestrus* spp. larval stages in Italy and considered three periods:

- a) an absolute prevalence of L1 which characterises the diapause (September–February)
- b) an increase in the percentage of L2 and L3 which corresponds to the active portion of the endogenous phase (February–September)
- c) an increase of L1 which corresponds to the flying period of the adult stages (exit phase) (May–September).

In Niger, the same result was obtained, with the disappearance of third larval instars during winter leading to a hypothesis of the presence of a diapause of L3 outside the animal. Furthermore, this same feature was described for *R. purpureus* in Egypt, where parasites were absent during winter (3).

In Egypt, Hilali *et al.* (18) found that L1 was present throughout the year with two peaks, in January and June. The L1 moult to L2 in late February and March (1st peak) and in late July and August (2<sup>nd</sup> peak).

The L3 also had two peaks, in April and August. After that, the number of L3 was reduced, indicating the formation of pupae followed by emergence of the adult stage.

Based on the findings reported herein, studies previously discussed and the work of Zumpt (2), a global interpretation of the dynamics (larval stages, instars/adults) of *R. usbekistanicus* in Tunisia can be proposed:

- a) a larval diapause from September to February, during which period only L1 are present;
- b) moulting of L1, which coincides with the most favourable environmental conditions (early spring) and is the beginning of an endogenous active phase, explaining the appearance of L3 during April;
- c) the adult free stage from May to September. The absence of L3 from May corresponds to the end of the active phase of the endogenous cycle.

In contrast with other countries, in Tunisia the absence of larvae lasted only one month, although this may be explained by the small number of animals examined during this period ( $n = 26$ ). There may be a second diapause in summer which has not been highlighted in this study.

In this survey, the overall intensity of infestation was 22.84 larvae per donkey. This result was similar to the finding of Otranto *et al.* (9), who found an intensity of 23.2 in Sicily (Italy). Other authors have found lower intensities; for example, Mula *et al.* (16) reported an intensity of infestation of 16.09 larvae per horse in Italy. Otranto *et al.* (9) found an intensity of 7.15 larvae per horse in Apulia (southern Italy).

In this study, two peaks of infestation were observed, the first during December (63.5 larvae per infested donkey) and the second in February (39 larvae per infested donkey). In contrast, in Egypt, Zayed *et al.* (3) reported a first infestation peak in March (38.92 larvae

per infested donkey) and a second one in June (57.86 larvae per infested donkey). Otranto *et al.* (9) noted the presence of L3 for four months (from April to July), with a peak in May (10.2 per infested horse) in the Apulia region (Italy). In Sicily, larvae were found for three months: April, July and August, with a peak in July (36 larvae per infested horse). In Sardinia, Mula *et al.* (16) found two infestation peaks, in March and June, with 48.17 and 24.76 larvae per infested horse, respectively. Hilali *et al.* (18) showed that L1 were present during all months of the year, with an infestation peak (intensity of 158 larvae) in January and a second peak in June; L2 were present only from February to April and in July and August, with two peaks, in March and August. Finally, L3 were present from March to May, and in August and September. Two peaks were also observed for L3 (April and August).

The overall abundance of infestation in this study was 12.06 per donkey. This abundance is slightly lower than that reported by Zayed *et al.* in 1992 (15.98 larvae per donkey examined) (14). The monthly kinetics of abundance follows that of intensity and shows the same peaks. Table IV summarises other studies compared with this work.

**Table IV****Studies of *Rhinoestrus* cited in this work**

Continent	Country	Animals (N)	<i>Rhinoestrus</i> species	Infestation prevalence (%)	Infestation intensity	Infestation peaks	Reference
Europe	Apulia region (Italy)	Native horses (212)	<i>Rhinoestrus purpureus</i> and	6.13	7.15	May (L3)	9
	Sicily (Italy)	Native horses (120)	<i>Rhinoestrus usbekistanicus</i>	4.16	23.20	July (L3)	
	Sardinia (Italy)	Horses (265)	<i>Rhinoestrus usbekistanicus</i>	49	16.09	June (L3)	16
	Apulia region (Italy)	Horses (212)	<i>Rhinoestrus</i> spp.	6.13	N/A	N/A	20
	Turkey	Horses	<i>Rhinoestrus</i> spp.	100	N/A	N/A	19

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Africa	Dakar (Senegal)	Donkeys (138)	<i>Rhinoestrus usbekistanicus</i>	84	15.90	N/A	4
	Niger	Donkeys (234)	<i>Rhinoestrus usbekistanicus</i>	8.10	N/A	November (L3)	8
	Giza governorate (Egypt)	Donkeys (144)	<i>Rhinoestrus purpureus</i>	66.11	23.31	March and August (L3)	3
	Giza governorate (Egypt)	Donkeys (144)	<i>Rhinoestrus purpureus</i> and <i>Rhinoestrus usbekistanicus</i>	100	N/A	January and August (L3)	18
	Fayoum, Beni- Suef and Monofia governorates (Egypt)	Donkeys (80)	<i>Rhinoestrus purpureus</i> and <i>Rhinoestrus usbekistanicus</i>	50	N/A	March (L3)	17

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N/A: not available

The clinical manifestations of rhinoestrosis vary from asymptomatic to deaths in horses and donkeys, with reports of nervous signs characteristic of encephalomyelitis after penetration of the larvae through the ethmoid bone and the soft cerebral membranes (2). In fact, this myiasis, which is characterised by irritation of the nasal cavities, sinuses and pharynx, can also cause lesions of the upper respiratory tract and lungs, with mild exudative inflammation, emphysema, interstitial pneumonia and eosinophilic infiltration (5). The mortality rate associated with this parasite was estimated to be 82% in horses from 27 farms in Russia (7).

A study conducted in Egypt showed that *Rhinoestrus* spp. induced anaemia in donkeys and that both inflammatory and immune responses against infestation were activated, with a parallelism between them in blood and nasal fluid. Blood loss and impaired appetite lead to decreased serum protein, including globulin, concentrations. The serum calcium level decreases in infested animals and oxidative stress may lead to problems with bone formation (17).

To the authors' knowledge, this is the first study to report the infestation of Tunisian equids by *R. usbekistanicus*. The results show the importance of the infestation of donkeys with *R. usbekistanicus* larvae in Tunisia. This will require the establishment of a veterinary intervention programme to minimise the impact of this parasite on animal health and welfare. Given that the highest prevalence of infestation occurs between September and November, systematic treatment of all equids with ivermectin or doramectin could be implemented in Tunisia.

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