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Low host specificity of *Hippobosca equina* infestation in different domestic animals and pigeon

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ABSTRACT

This study aimed to highlight the low host specificity of *Hippobosca equina* (*H. equina*) that poses a danger in diseases transmission between different animal species as well as, identification of the collected flies using light microscope and molecular characterization of *H. equina* in Egypt. Two hundred and forty flies were collected weekly from different animal species from El-Faiyum, Al Qalyubia and Kafr El-Sheikh Governorates, Egypt at the period from May to September of 2020. Insects were phenotypically and genetically identified then classified into 170 (70.8%) males and 70 (29.2%) females. The highest prevalence of *H. equina* was noticed from mid-June to the end of August. The sequencing of COI gene of five *H. equina* fly collected from different hosts as (horse, pigeon, cattle, buffalo, and donkey) were submitted to the GenBank under the accession numbers of MZ452239, MZ452240, MZ461943, MZ461944, and MZ461945, respectively. For insect infestation control, fipronil and deltamethrin is monthly sprayed for animals, birds and their circumference give a best result in our control study in the field. Deltamethrin showed a success in the elimination process and control measures of external parasites of pigeon.

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1. Introduction

Hippoboscidae are an obligate blood sucking arthropod that infest a variety of worm blooded animals (horse, donkey, cattle, buffalo, camel, red deer, dogs, and hares) as well as avian species and sometimes attack people (Andreani et al., 2019; Andreani et al., 2020; Bezerra-Santos and Otranto, 2020). The Hippoboscidae flies, sometimes known as “louse flies”, “forest flies” or “keds” and as a result to its very strong exoskeleton the insect is named also the “iron fly” (Rahola et al., 2011; Sokół and Michalski, 2015). They cause many health implications as they act as vectors for different

blood parasites (*Bartonella* spp., *Besnoitia besnoiti* and *Corynebacterium pseudotuberculosis*) to humans and animals, playing a role in mechanical transmission of different diseases and cause skin injuries, host irritations due to their strong painful bites which sometimes resulted in anaphylactic reaction (Arafa et al., 2019; Oboña et al., 2019; Andreani et al., 2020; Salem et al., 2022). Infested animals rub against various hard objects (walls, trees, fences, trunks), and move their tail and limbs nervously which may result in leg fracture due to vigorous movement especially in horses (Sokół and Michalski, 2015). Adults of both sexes are obligate blood sucking, and they can harbor a variety of infectious agents, such as bacteria, protozoa, helminths, and certain viruses (Halos et al., 2004; Liu et al., 2016; Skvarla and Machtinger, 2019). The Hippoboscidae family is subdivided into three subfamilies: Hippoboscinae, Ornithomyiinae and Lipopteninae which including 213 different species (Dick, 2006; Rani et al., 2011; Oboña et al., 2019). Some species are host-specific, whereas others infest a wide range of hosts (Ibáñez-Bernal et al., 2015; Mehlhorn, 2016; Veiga et al., 2018). The female insects are macro-larviparous,

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as females keep the larva in their uterus until the end of the third instar; the three larval instars feed on the milk glands then pupation occurs rapidly after pre-pupae laying (Mehlhorn, 2016). The larva (or pupa) is deposited in birds' cages and feathers or on the skin of a mammalian host (Halos et al., 2004). Adult insects increase in summer and autumn (Sokół and Michalski, 2015). Regular usage of safe acaricides is recommended for external parasites control, Fipronil is a phenyl pyrazole broad spectrum insecticide that has been approved for use in agriculture and livestock external parasites control in several countries (Kitulagodage et al., 2011). Fipronil is used for control of ants, lice, beetles, flies, fleas, ticks, mites, termites, cockroaches, mole crickets, thrips, rootworms, weevils, and other insects (Jackson et al., 2009). Although, Fipronil is an effective insecticide, but it is very toxic for honeybees, some birds, fish, shrimp and aquatic insects (Jackson et al., 2009). Deltamethrin is a synthetic ester pyrethroid insecticide that was first introduced to the market in 1978 after being initially reported in 1974. Deltamethrin can kill insects directly via disrupting the nervous system's regular function of parasites and insects (Johnson et al., 2010). Deltamethrin is an effective insecticide which has a wide safety margin for mammals and birds (Elias, 2013; Salem and Attia, 2021) but it is highly toxic for fishes, aquatic insects and honeybees (Chandra et al., 2013). So, this study aimed to pick up the louse fly from different sources (horse, donkey, cattle, buffalo and pigeons) and identify it under light microscope with molecular characterization using PCR as well as sequence analysis; in addition to application of suitable insecticide to overcome the hazard effect of annoying louse fly.

2. Material and methods

2.1. Sample collection

Samples were weekly collected between May to September of 2020, two hundred and forty samples of *H. equina* were captured and examined from different farms from: El-Faiyum Governorate; located about 81 mi southwest of Cairo, Egypt. The climate of the region is very hot in summer season as the ambient temperature ranged from 35.5 °C (95.9°F) to 39 °C (102.2°F) with relative humidity (36 to 44%), Al Qalyubia governorate; found about 30.41°N 31.21 °E with ambient temperature range from 35.9 °C (96.7°F) to 36.4 °C (97.4°F) with relative humidity (45% to 53%) and Kafr El-Sheikh lies in (31.3°N 30.93°E) with ambient temperature range 31.6 °C (88.9°F) to 33 °C (91°F) with relative humidity (54% to 62%).



Fig. 1. Mixed rearing in the same farm: note; pigeon house beside dairy cattle yard.

The investigated farms usually include mixed rearing (Fig. 1) and insects were collected as follow, 40 insects from cattle (*Bos taurus*), 40 from buffalo (*Bubalus bubalis*), (40) from horse (*Equus caballus*), (40) donkey (*Equus asinus*) and (80) from pigeon (*Columbidae*). Each sample of *H. equina* was manually collected from the host and kept into separate, labeled Eppendorf tubes with 70% ethanol then transported for further investigations to the Laboratory of the Parasitology Department; Faculty of Veterinary Medicine; Cairo University.

2.2. Ethical statement

Animals and birds were humanly examined for the Presence of *H. equina* following the rules of Research Ethics Committee of faculty of Veterinary Medicine Cairo University. Then, all investigated animals and birds were released securely at the examination site without any hurt.

2.3. Parasitological examination

Insect samples were investigated under stereoscopic Microscope (Olympus; CX33, Japanese) to detect and photographed each part of the louse fly according to Salem and Attia (2021).

2.4. Molecular identification

DNA was extracted using the DNA quick kit (Qiagen, Santa Clara, CA, Germany) according to the manufacturer's instructions, except the elution amount was 30 ml, or in certain cases, The exoskeleton was punctured by CTAB (Cetyl Trimethyl Ammonium Bromide) phenol/chloroform extraction technique while keeping the specimen's exoskeleton as a voucher. A 760 bp of the COI gene (also known as "rudimentary" in *Diptera*) was amplified and sequenced following the method of Moulton and Wiegmann (2004); Attia and Salem (2021).

The selected primers used in this reaction were recorded in Petersen et al. (2007) and Attia and Salem (2021).

The thermal cycling program was summarized as PCR cycling protocol targeting the COI gene was proceeded as follow; the initial denaturation step was at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 40 s, annealing at 51 °C for 70 s and elongation at 72 °C for 2 min followed by the final extension step at 72 °C for 10 min. The target amplified product size expected at 760 bp. On the gel after electrophoresis. The sequences were reviewed and modified manually with BioEdit software as described by Abu-Elala et al., (2018). The obtained gene sequences of *H. equina* were then matched with other related homologous genes found in the GenBank database using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) following the approach outlined by Abdelsalam et al., (2020).

2.5. Insect control

Application of both Fipronil and Deltamethrin as insect control tool were followed as follow; Fipronil 0.25 percent solution (Frontline; Merial), (1 ml/10 kg body weight) through pour on animals back is recommended for monthly application. Deltamethrin (Butox, 12.5% solution, 1 ml/4 L of water for pigeons & 1 ml /L for surrounding environment), is recommended for monthly spraying. Precautions should be taken when using fipronil and deltamethrin through avoidance the direct contact with the skin & face of operators as well as animal food and water. The amount of diluted solution should be adjusted and in case of presence of a residual amount, it should be hygienically disposed with its containers after usage.

2.6. Data analysis

Data were stated as means \pm standard errors. The data were analyzed statistically using SPSS Inc., Chicago, IL, USA; Version 18.0 software.

3. Results

3.1. Clinical signs on investigated animals and birds

Cattle, buffaloes, donkeys, and horses infested with *H. equina* showed signs of restlessness, vigorous movement of the tail and irritability. Pigeons showed signs of irritability, itching, ruffled feathers, weight loss and emaciation.

3.2. Distribution of *H. equina*

Hippobosca equina is commonly distributed in cattle and buffaloes: around anus, around external genital organ (vagina), udders, body, face, and then inner aspect of thighs (Fig. 2). In horses & donkeys, *H. equina* is commonly demonstrated in horse around anus & genital organs as well as the inner aspects of thighs (Fig. 3). While in pigeons *H. equina* is commonly observed on and under wings, body, and tail (Fig. 4).

The *H. equina* intensity (burdens abundant) was (5–100) in cattle, (1–80) in buffalo, (1–25) in horses, (5–60) in donkeys and (2–6) in pigeons. The highest prevalence of *H. equina* was observed from mid-June to the end of August as seen in (Table 1).

3.3. Morphological identification of *H. equina*

Two hundred and forty *H. equina* fly were subdivided into 170 (70.8%) males and 70 (29.2%) females.

H. equina (Diptera: Hippoboscidae), had length ranged from 6.5 to 9 mm (7.5 mm \pm 0.5). The body is reddish brown in color or even leathery with sac like abdomen. Its wing is large hyaline and

extend beyond the body margin. The wing venation is characteristic which has 7 long vein and two cross one. The body supported with bands and dense long setae. The antennae are housed in deep hollows called antennal sockets. The legs are long and strong for adaptation to climb and attach well on the hosts (Fig. 5). The differentiation of sex through presence of vaginal opening in female and aedeagus in male (Fig. 6).

3.4. Sequencing and phylogenetic analysis

The sequenced COI gene of five horse louse fly collected from horse, pigeon, cattle, buffalo, and donkey hosts were submitted to GenBank under the accession numbers MZ452239, MZ452240, MZ461943, MZ461944 and MZ461945, respectively. The five sequences displayed 100% identity with each other. All sequences showed 100% similarity with the COI gene of *H. equina* (MW590980.1-Finland, and MK737646.1-Egypt), 99.85% similarity with the COI gene of *H. equina* (MN868873.1-Portugal), 99.83% similarity with the COI gene of *H. equina* (EF531208.1-Denmark), 96.48% similarity with the COI gene of *H. longipennis* (MK405667.1-Czech Republic), this result showed that these specimens are deeply embedded in the genus hippoboscidae. The identities of these sequences were confirmed as *Hippobosca equina*. Interestingly, phylogenetic analysis of the COI gene sequences revealed two major lineages (Fig. 7). The first major clade comprises two subclades: the first includes the genus *Hippobosca*, the second subclade with strong nodal support consists of *Lipoptena*, and *Olfersia*. The second major clade is consisting of *Haematobia* and *Blondeliini* species (Fig. 8).

3.5. Insect control

The regular monthly spraying of fipronil 0.25 percent solution (Frontline; Merial) with controlled management and hygiene, there was a marked decrease in the insects count and number. Proved that deltamethrin showed a success in the elimination process of external parasites of pigeon.

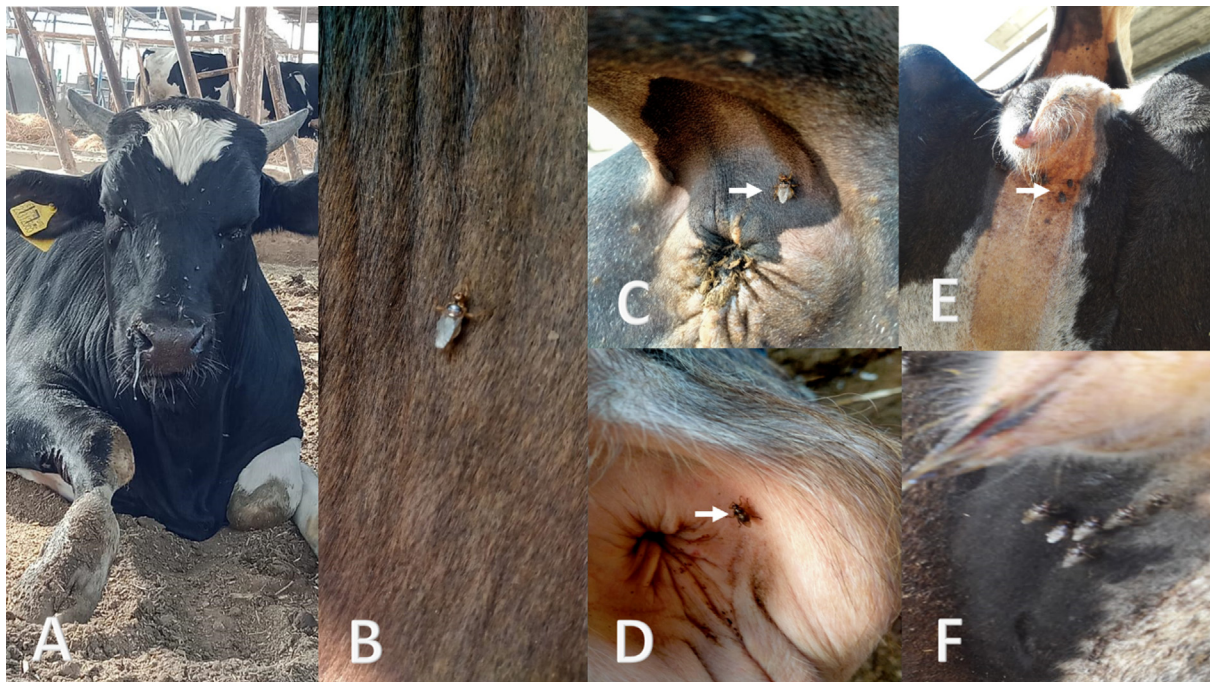


Fig. 2. A: *H. equina* on cattle face. B: *H. equina* on cattle skin. C: *H. equina* observed around the anus of cattle. D: *H. equina* observed around the anus of buffalo. E: Three *H. equina* observed around the vagina of cattle. F: Six *H. equina* on cattle skin.

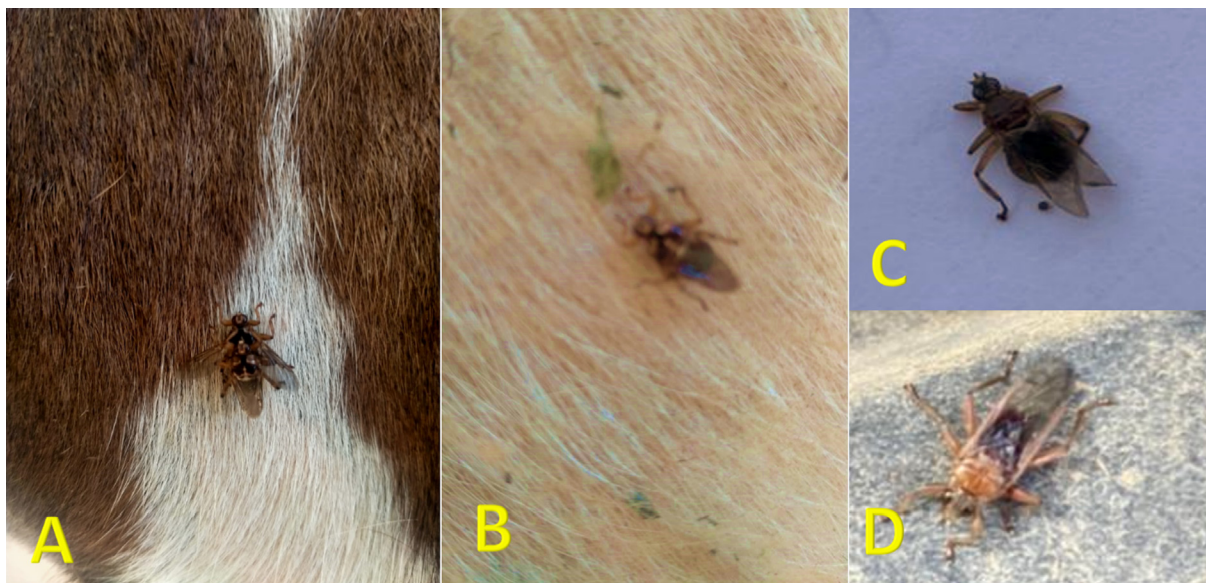


Fig. 3. A: *H. equina* observed on horse skin. B: *H. equina* observed on donkey skin. C&D: Adult *H. equina* fly.

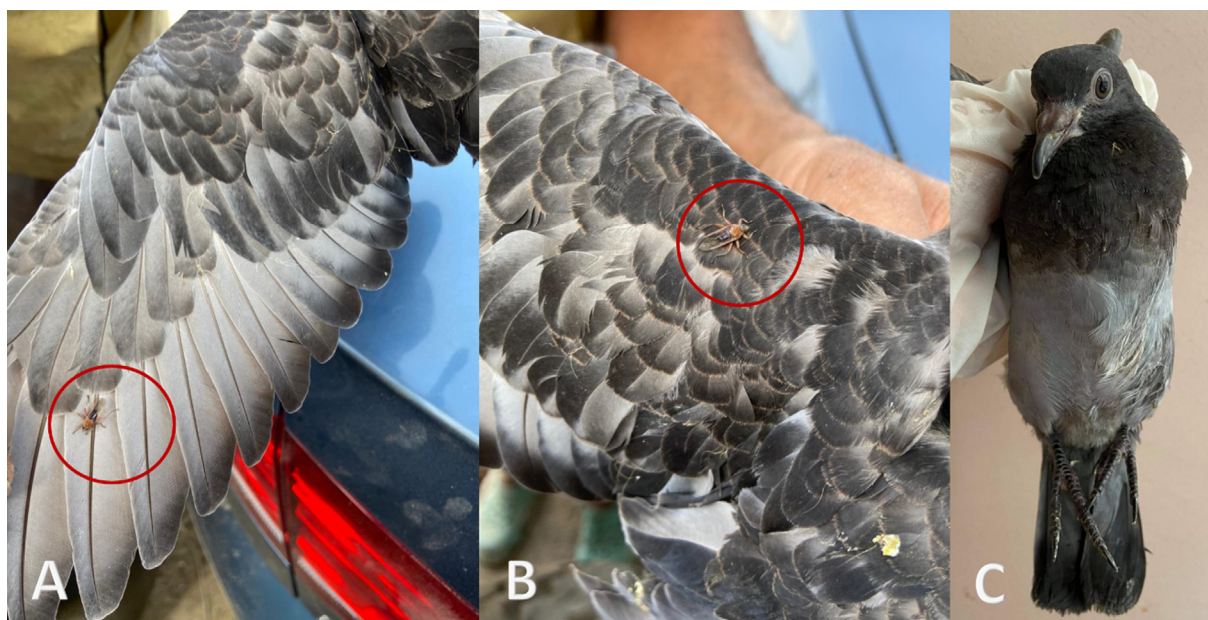


Fig. 4. A&B: *H. equina* observed on pigeon wing. C: Pigeon showed ruffled feather and emaciation.

Table 1
The intensity of (*H. equina*) in different species.

Governorate	El-Faiyum					Al Qalyubia					Kafr El-Sheikh				
	Horse	Donkey	Cattle	Buffalo	Pigeon	Horse	Donkey	Cattle	Buffalo	Pigeon	Horse	Donkey	Cattle	Buffalo	Pigeon
Examined animals & birds															
May	5 ± 1	5 ± 3	18 ± 1	8 ± 4	2 ± 3	3 ± 1	5 ± 1	10 ± 3	6 ± 5	3 ± 1	1 ± 1	8 ± 1	5 ± 1	1 ± 1	2 ± 1
June	23 ± 2	44 ± 1	45 ± 2	23 ± 4	3 ± 1	14 ± 2	30 ± 3	23 ± 1	30 ± 4	4 ± 1	19 ± 4	29 ± 4	33 ± 3	36 ± 3	3 ± 1
July	20 ± 1	45 ± 3	38 ± 1	22 ± 1	2 ± 1	10 ± 1	36 ± 2	20 ± 2	27 ± 3	3 ± 2	13 ± 3	22 ± 3	36 ± 5	30 ± 5	3 ± 2
August	25 ± 3	60 ± 2	100 ± 1	80 ± 4	6 ± 3	12 ± 2	48 ± 1	50 ± 2	46 ± 2	3 ± 1	10 ± 4	35 ± 3	90 ± 6	45 ± 4	4 ± 2
September	19 ± 1	45 ± 2	78 ± 3	34 ± 1	5 ± 1	9 ± 3	30 ± 1	35 ± 3	35 ± 1	2 ± 2	8 ± 3	28 ± 4	40 ± 5	20 ± 3	2 ± 1

*: The average number of insects that observed in (20 animals/species/month) (5 animals/species/week). Data expressed as mean ± Standard errors.

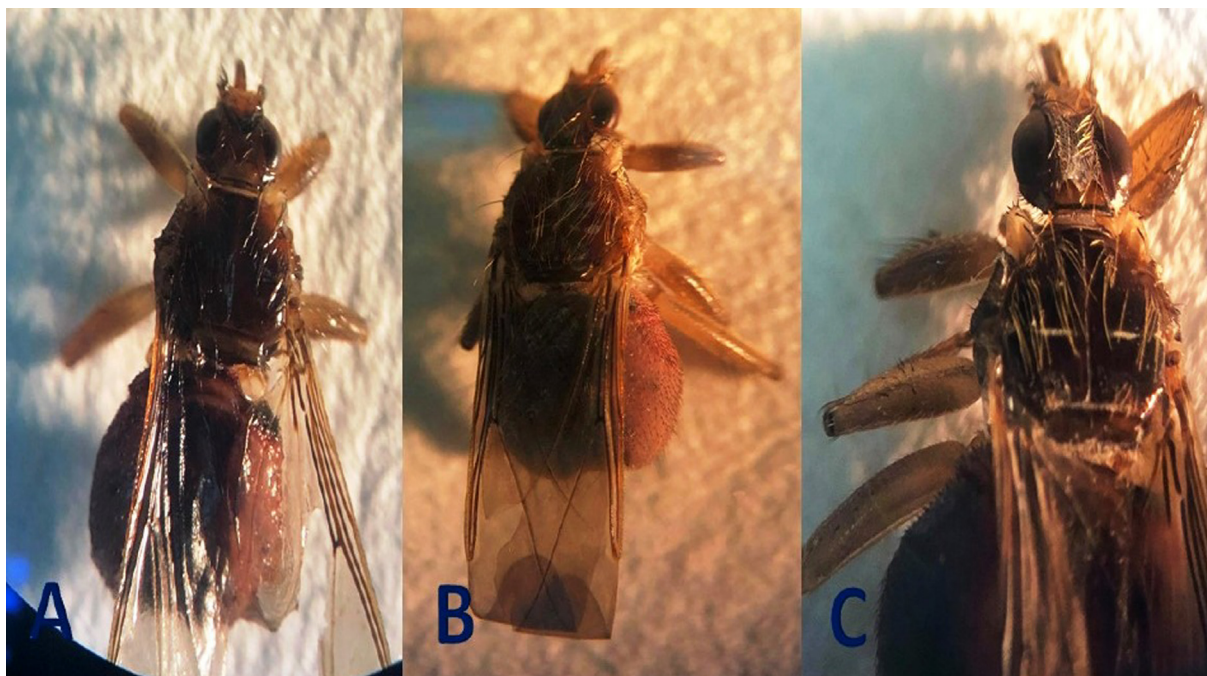


Fig. 5. Dorsal aspect of fly. **A:** Light microscopic observation of adult *H. equina* showing that the body is reddish brown in color or even leathery with sac like abdomen. **B; C:** Its wing is large hyaline and extend beyond the body margin; The wing venation is characteristic which has 7 long vein and two cross one.



Fig. 6. A: Female *H. equina* fly showing vaginal openings (arrow) B: Male *H. equina* fly showing the aedeagus (arrow).

4. Discussion

External parasites are widespread and abundant. *H. equina* flies are a worldwide annoying hematophagous insect that infest a diversity of animal species. This study aimed to confirm the low host specificity of the louse fly, *H. equina*, that acts as a potential intermediate host most of blood parasites and it can transmit them biologically and mechanically between different warm-blooded animals and sometimes, they attack humans also, insects considered as stress factor (Halos et al., 2004). So, biosecurity measures, strict hygiene, periodical usage of environmentally safe insecticide (El-Saadony et al., 2020; Saad et al., 2021), and improvement of the

animal and bird's general health should be adopted via using of natural safe preparation such as prebiotics (Yaqoob et al., 2021; Abd El-Hack et al., 2021a), probiotics (Alagawany et al., 2021a; El-Saadony et al., 2021a; Abd El-Hack et al., 2021b), bioactive peptides (El-Saadony et al., 2021b; El-Saadony et al., 2021c), herbal extracts (Abou-Kassem et al., 2021; Abd El-Hack et al., 2021c; Saad et al., 2021), Phytogetic feed additives in animals diets (Abdel-Moneim et al., 2022), essential oil (Alagawany et al., 2021b; El-Tarabily et al., 2021), green synthesized nanoparticles (El-Saadony et al., 2021d) organic acids, ...etc. should be taken into consideration by farmers and veterinarians to avoid such problems. From our results *H. equina* is annoying ectoparasites

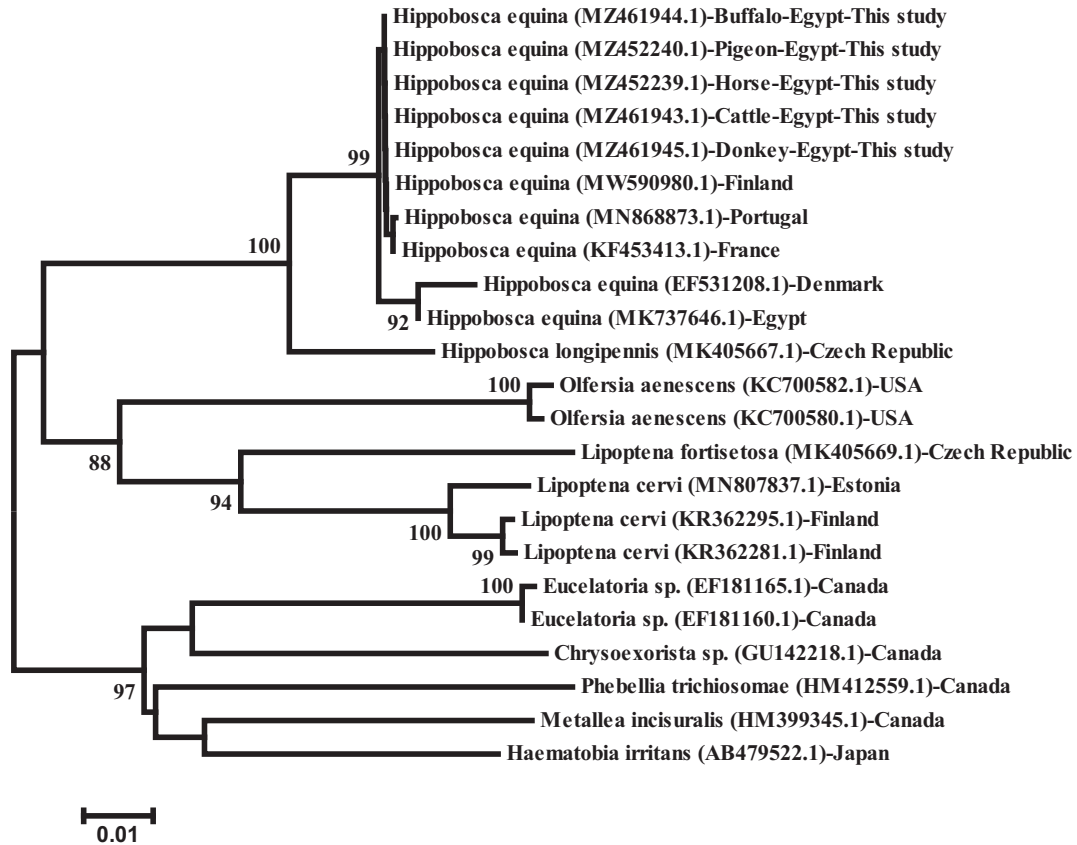


Fig. 7. Phylogenetic tree based on maximum composite likelihood model for the neighbor-joining method for the present of *H. equina* and another related superfamily retrieved from GenBank.

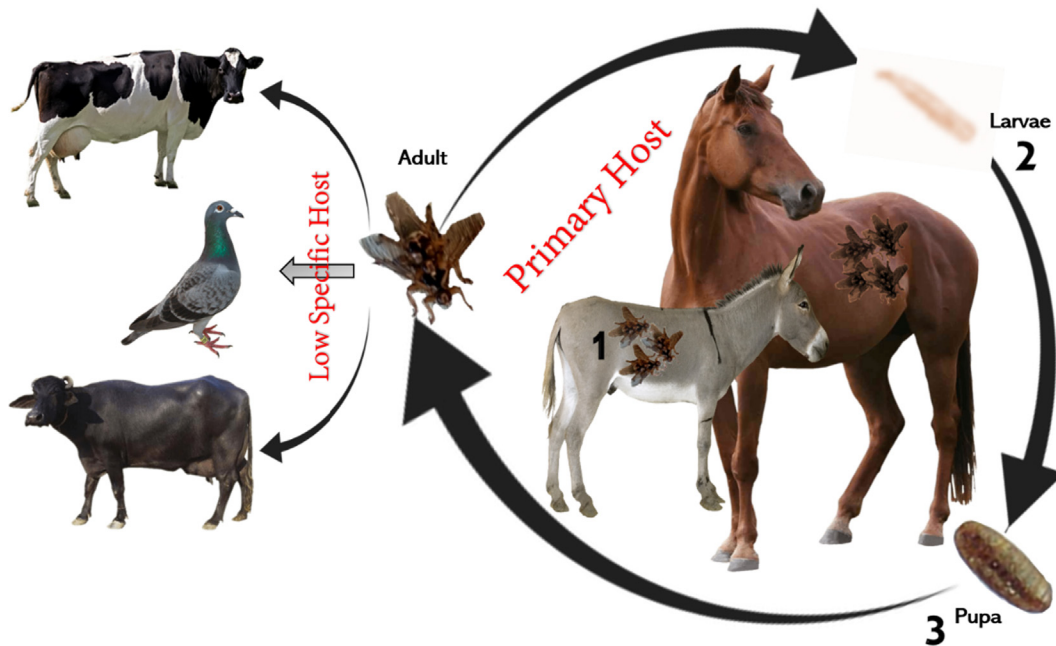


Fig. 8. Life cycle of *H. equina* showing adult winged flies lay larvae in the surrounding environment, which immediately pupate and gives a new winged adult. The figure describes also, the low host specificity of *H. equina* that attack different animals (horse, donkey, cattle, and buffalo) as well as, pigeons.

causing restlessness for cattle, buffalo, horse, and pigeons. This finding is agreed with that mentioned by Jozef et al., (2019). The highest prevalence of *H. equina* was recorded from mid-June to

the end of August. This finding may contribute to the high temperature that recorded in these months, this result is parallel to that recorded by Sokół and Michalski (2015) as they found that the

highest prevalence of *H. equina* in Polish primitive horses was observed from mid-June to the end of July in Poland. Also, Syame et al., (2008) and Zittra et al., (2020) observed that the higher activity of *H. equina* in the summer months. The intensity of *H. equina* was 5–100 in cattle, (1–80) in buffalo, (1–25) in horses, (5–60) in donkeys and (2–6) in pigeons. Reviewing the available literature, no available recent data about *H. equina* in different animal spp. were found.

From our observation, Hippoboscids are a low host specific insect, *H. equina* fly infest a wide range of hosts (cattle, buffalo, horse, donkey, and pigeons) but, this finding is completely disagreed with Rani et al., (2011) as they described Hippoboscids as a highly specific larviparous ectoparasitic flies that spend their entire adult lives in the fur or feathers of their mammalian and avian hosts. On the other hand, Quercia et al., (2005) confirmed the low host specificity of the insect and perform the first clinical report of an allergic reaction in female farmer after the bite of *Hippobosca* spp. Also, Sokół and Michalski (2015) confirmed the low host specificity of the insect and reported that *H. equina* could attack cattle, dogs, hares, birds, and humans. Hafez et al., (2009) recorded *H. equina* infestation in cows, buffaloes, donkeys, and camels in Egypt.

From our observations, *H. equina* was found in around anus, around external genital organ, udders, body, face, and then inner aspect of thighs. In horses & donkeys, *H. equina* was observed in horse around anus & genital organs as well as the inner aspects of thighs. While in pigeons *H. equina* was noticed in wings, under wings, body, and tail. Similar findings were recorded by Sokół and Michalski (2015) as they found that *H. equina* infest the sensitive skin: around the anus, perineum, and the inner aspect of the thighs in horses, under the lower part of the valvular labia and under the tail in cattle.

Owing to our results, the number of male insects was 170 (70.8%), while the female number was 70 (29.2%). On another study, Boucheikhchoukha et al. (2019) collected 29 *H. equina* from horses from the El Tarf barns in northeastern Algeria and classified them into (14 males and 15 females). The difference in male to female ratio may be contributed to the differences in localities, ambient temperature, environmental and managemental conditions in which sample were collected, as well as the number of examined insects.

Phylogenetic analysis revealed monophyly among Hippoboscoidea members, implying that the superfamily's ancestor was a free-living fly that fed on blood of mammals (Dittmar et al., 2006; Petersen et al., 2007).

From our results the sequenced COI gene of five horse louse fly collected from horse, pigeon, cattle, buffalo, and donkey hosts with the accession numbers MZ452239, MZ452240, MZ461943, MZ461944 and MZ461945, respectively were displayed 100% identity with each other. All submitted sequences showed 100% similarity with the COI gene of *H. equina* (MW590980.1-Finland, and MK737646.1-Egypt), 99.85% similarity with the COI gene of *H. equina* (MN868873.1-Portugal), 99.83% similarity with the COI gene of *H. equina* (EF531208.1-Denmark), 96.48% similarity with the COI gene of *H. longipennis* (MK405667.1-Czech Republic), as seen in (Fig. 7). This finding is correlated to the study of Pohjoismäki and Kahanpää (2014) and parallel to the checklist of the superfamilies Hippoboscoidea in Finland (Oboňa et al., 2019).

Strategies for insects' control are significantly difficult to veterinarians because of problems that arises due to the improper use of insecticides, which may result in acquired insect resistance to insecticides, toxicity to the host and/or handlers as well as environmental contamination (Benelli and Duggan, 2018; Benelli and Pavela, 2018a; Benelli and Pavela, 2018b; AbdElKader et al., 2021). In this study, monthly application of fipronil succeeded in limitation and control of *H. equina* in animals. This result agrees

with Rendle et al., (2007) as they found that fipronil is an effective insecticide against *Chorioptes* species mites in equine. On the other hand, Kitulagodage et al., (2011) found that oral administration of single dose of fipronil has a toxic as well as residual effects in northern bobwhite quail. So, from our finding we recommend the proper usage of fipronil according to instructions for use to avoid its negative effect. Due to the toxic effect of fipronil on birds and its persistence in the surrounding environment (Kitulagodage et al., 2011), deltamethrin was selected for insect control for pigeons and surrounding environment. From our observations, deltamethrin succeeded in insect control in pigeons and surrounding environment. This result is agreed with that recorded by Elias (2013); Eladl et al. (2018); Attia and Salem (2021), Salem et al. (2022).

5. Conclusion

The current study is proved that *H. equina* is low host specific fly and play a role in both biological and mechanical diseases transmission between different animals' species. Intermingled and mixed rearing system closely between different animal species is not recommended and should be avoided. Also, restricted & regular insect control measures using different molecules should be adopted to avoid the hazard effect of insect infestation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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