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#### Chapter

# Spinose Ear Tick *Otobius megnini* Infestations in Race Horses

Rupika S. Rajakaruna and Chulantha Prasanga Diyes

## Abstract

Spinose ear tick, *Otobius megnini*, has a worldwide distribution causing otoacariasis or parasitic otitis in animals and humans. It mainly infests horses and cattle. It is a nidicolous, one-host soft tick spread from the New World to the Old World and is now distributed across all the continents. Only the larvae and nymphs are parasitic, feeding inside the ear canal of the host for a long period. Adult males and females are free-living and nonfeeding, and mating occurs off the host. Being inside the ear canal of the host allows the tick to be distributed over a vast geographic region through the distribution of the host animals. The presence of infectious agents *Coxiella burnetii*, the agent of Q fever, spotted fever rickettsia, *Ehrlichia canis*, *Borrelia burgdorferi*, and *Babesia* in *O. megnini* has been reported, but its role as a vector has not been confirmed. Human infestations are mostly associated with horse riding and farming through close contacts with companion animals. Control measures involve use of acaricides, repellants, and biological control methods. However, controlling the tick population and its spread is extremely difficult due to its life cycle pattern, seasonal dynamics, and resistance to certain acaricides.

Keywords: Otobius megnini, spinose ear tick, horses, otoacariasis

#### 1. Introduction

The spinose ear tick, Otobius megnini (Dugès 1883) (Acari: Ixodida: Argasidae), is an economically important soft tick as it parasitizes livestock mostly cattle, goats, sheep, and horses and also infests humans [1–7]. Otobius megnini is a one-host soft tick from the New World with a wide geographical distribution. Its original center of distribution is considered to be the southwestern North America from where it spread to Central and South America [5, 8]. Since the larva and the nymph of this tick feed inside the ear canal of the host for a long period, it allows the tick to be distributed over a vast geographic region transcontinentally through the distribution of the host animals. It has distributed far north as Canada where it is reported southeastern parts of British Columbia infesting mountain goats, mountain sheep, mule, white-tailed deer, elk, cattle, and a house cat [9]. Two scenarios have been put forward to explain how the tick reached the Old World: (1) During the Boer Wars (in the late nineteenth century between the United Kingdom and the Boers of the South African Republic), the movement of horses bringing the tick from South America or Mexico to South Africa and (2) after the Boer Wars, importation of cattle from the United States to South Africa [5]. From there it was introduced to many neighboring countries in the African continent: Madagascar, Lesotho,



Figure 1.

Geographic distribution of spinose ear tick Otobius megnini (modified from Keirans and Pound [5] with permission).

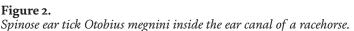
Botswana, Namibia, Zimbabwe, Zambia, Malawi, Nigeria, and Democratic Republic of Congo [5]. The first report of *O. megnini* from Europe was in 1901 from a US resident who visited the UK with a tick in the ear [10], and there is another unverified report of O. megnini from Denmark [11]. Dogs imported from South Africa to Italy [12] and to Sweden carried the tick in the ear canal [13]. In Turkey, it was first recorded in 1988 [14], and it is well established now [15–17]. Otobius meg*nini* is thought to have reached India in the mid-1930s together with cattle or horses brought from Southern Africa. This tick species is recorded in race horses brought from farms in northern India for an auction at the Madras Race Club [18]. There is a speculation that O. megnini was introduced to Sri Lanka from India via horse trading. The first report of *O. megnini* in Sri Lanka is in 2010 from stable workers and jockeys as an intra-aural infestation [7, 19]. In Sri Lanka, this tick appears to have a limited distribution with no records of it infesting any other domesticated animals other than horses in the racecourses. It has now moved to Far East as Korea [20] and Western Australia [21] and has been recently reported from Iran [22]. Figure 1 shows the current geographic distribution of O. megnini in the world.

The presence of *O. megnini* inside the ear canal is known as otoacariasis or parasitic otitis. It can cause toxic conditions, allergies, paralysis, muscle spasms, irritations, eardrum perforation, and myotonia, and *O. megnini* has been listed as a potential vector for many tick-borne infections [23–25]. Studies have reported deaths of domestic cattle and horses as a result of heavy infestation of *O. megnini* [9, 26–29].

#### 2. Horse otoacariasis

Otobius megnini is the causative agent of horse otoacariasis. This condition can cause serious injury and occasionally death in horses [9, 30], and common clinical signs include abnormal head carriage, head shaking, and head rubbing [31]. Early studies report nervous disease [32] and auricular nerve paralysis [33] due to the presence of *O. megnini* in the ear canal of horses. Intermittent painful muscle





cramps not associated with exercise were described in horses that were severely infested with *O. megnini* [34]. Infested horses show cramping of pectoral, triceps, and abdominal muscles lasting from minutes to a few hours with severe pain that often resembles colic [34]. Between muscle cramps, horses appear to be normal, and once the ticks are removed, clinical signs are reduced and recovered within 12–36 h [34]. While a neurological pathology that includes muscle tremors and muscle contractions are observed, electromyographic measurements suggest these may be due to increased motor unit activity [34]. No conclusive evidence supports the classification of *O. megnini* as a paralysis tick. The fact that this tick feeds within the ears of its hosts where inflammatory reactions could affect the balance of the host and lead to symptoms that could be interpreted as being neurological in origin should be considered. Recently, from Northern Mexico, a 2-year-old quarter breed was reported having myotonia and colic associated with the infestation of *O. megnini* [35]. **Figure 2** shows larvae and nymphs of *O. megnini* inside the horse ear canal.

#### 3. Life cycle

The life cycle pattern of soft ticks varies considerably among the populations of the same species as well as between species of the family *Argasidae*. *Otobius megnini* has four stages in its life cycle: egg, six-legged larva, nymph, and adult. The number of nymphal instars in the life cycle varies and is controversial. Studies have reported the presence of either one [6, 36, 37] or two nymphal stages [5, 20, 38] and also the presence of a third nymphal instar [39]. Unfed larval stage is highly active showing constant and rapid movements. Nymphs have distinct integument covered with short blunt spines and hence the name spinose ear tick. The adults have spineless-granulated integument. Only the larvae and nymphs are parasitic and stay attached inside the ear canal for extended periods of time, while the adult is a nonfeeding free-living stage [40]. The life cycle pattern of *O. megnini* closely resembles that of a one-host hard tick by having a long parasitic period and a short nonparasitic period.

The larvae and nymphs feed for several days to months [6, 38, 41, 42]. Fully engorged nymphs detach after a long parasitic phase, drop off, and molt on the ground to nonfeeding adults [42]. *Otobius megnini* has a single gonotrophic cycle; hence, females die soon after oviposition. Successful completion of the life cycle depends on the efficacy of the blood meal which is determined by the interactions with their host and environmental conditions [43, 44]. Temperature and humidity have been identified as the main climatic variables that contribute to the nature of the life cycle [45] on which the egg incubation and hatching success, larval and nymphal feeding, survival and pre-molting periods, and female oviposition and survival are dependent [42, 46–48]. Female ticks, compared to males, tend to take a larger blood [42, 49]. Under laboratory conditions, *O. megnini* can feed on rabbits and complete the life cycle successfully (**Figures 3** and 4) [42].

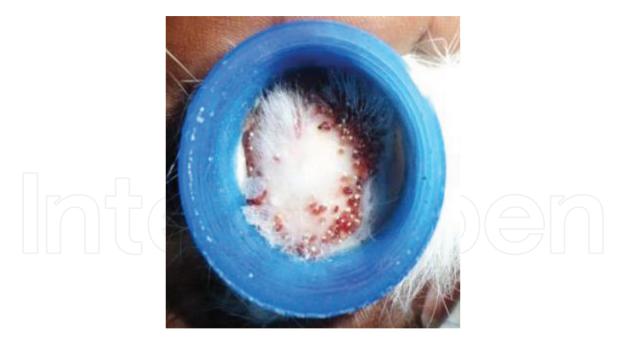
Ticks developing in temperatures between 21 and 28°C typically have oviposition 6–12 days after dropping as nymphs from their hosts. The number of eggs, which are laid in the nesting grounds of potential hosts, can range from 398 to 1187 depending on the weight of the female [40]. Egg incubation ranges from 14 to 19 days in laboratory studies [50] and 18–23 days in field studies [26]. Once hatching occurs, larvae seek hosts for survival; unfed larvae have been found to survive in the laboratory up to 78 days [51]. Larvae feed on the host for 1–5 weeks and then molt into the nymph. The majority of nymphs feed between 2 and 4 months [50] but some up to 6 months [51].

A tropical population of *O. megnini* successfully completed the life cycle within 123 days [42]. Only the larger larvae and nymphs weighing more than 0.9 mg molt to the next stage. Larvae do not molt if the temperature is below 10°C, and there is a higher survival of larvae at 28°C. Nymphs undergo diapause if the temperatures are below 10°C [42]. Females survive longer (313–629 days) than males (142–321 days). Some females lay eggs without mating. However, parthenogenesis is not confirmed. Apart from the descriptive study on Sri Lankan population of *O. megnini* [42], life history of laboratory populations of Nearctic population of *O. megnini* from the Southwestern USA (duration of the life cycle: 52–248 days; [52]); Texas, USA (92–125 days; [53]); California, USA (62–118 days; [50]); Maryland, USA (60–120 days; [54]); neotropical population of *O. megnini* from Córdoba, Argentina (101.4 days, [9]) and oriental population of *O. megnini* from Madras, India (69–98 days; [36]) and Bangalore, India (118–207 days, [38]) has been reported with considerable variations in parasitic period, molting period, fecundity, and survival. These variations can be attributable to the



#### Figure 3.

Life-history stages of Otobius megnini. (A) Eggs. Larvae: (B) unfed free-living larva soon after hatching, (C) Engorged larva. Nymphs: (D) unfed nymph soon after hatching inside the ear canal, (E) dorsal view of the engorged nymph fed on horse, (F) ventral view of engorged nymph, (G) nymph molting. Nonfeeding adults: (H) dorsal view, (I) ventral view of a female, (J) ventral view of a male, (K) female genital pore (L), and male genital pore (scale bar indicating 1 cm).



**Figure 4.** Larvae and nymphs of Otobius megnini feed on New Zealand white rabbits under laboratory conditions.

differences in laboratory conditions that the ticks were exposed and the host animals used to feed the immature *O. megnini* (e.g., rabbits, cattle). However, data obtained from these laboratory studies cannot entirely extrapolate to the natural context uncritically because the survival period of *O. megnini* greatly depends on their niche condition (e.g., air temperature, soil temperature, relative humidity and amount of direct light) [45] and the presence of pathogens, predators, and parasitoids [55]. The remarkable survival strategy of *O. megnini* has enabled the global expansion of this tick.

#### 4. Seasonal dynamics

*Otobius megnini* is adapted to survive in diverse ecological niches in tropics, subtropics, and temperate regions [6, 37, 41, 52]. In nidicolous ticks that have distinct seasonal activity, reproductive diapause with respect to temperature and photoperiod has been suggested as the main mechanism, which controls the seasonal activity. However, ambient climatic conditions needed for better survival of ticks greatly vary from region to region [41, 56, 57]. Therefore, there is a high geographic variation in seasonal population dynamics of ticks. In temperate region where distinct seasonal pattern is pronounced, the activity of different development stages of ticks is directly linked with the changing environmental condition. However, in the tropics, the altering of rainy and dry periods has been identified as main contributing factors of tick seasonal activity [41].

Studies conducted in Argentina [6, 58], South Africa [59, 60], and Texas, USA [61] have reported discordant results for seasonal activity for *O. megnini* indicating absence of a clear seasonal pattern. Further, climatic factors such as annual rainfall, temperature, and altitude appear to have no profound effect on distribution and seasonal dynamics of *O. megnini* populations in Argentina and the Union of South Africa [58, 60, 62]. In the tropics, the larvae show clear seasonal dynamics with a high larval activity during warmer and dryer months [42]. Information about seasonal dynamics of *O. megnini* is important in controlling populations because the sanitary measures and applications of biocides considering the seasonal dynamics have been able to minimize the tick abundance [63].

#### 5. Infectious agents

Since O. megnini is a one-host tick and the adult females do not feed, spreading of infectious diseases from one host to another is limited unless the infectious agent shows both transovarial and transstadial transmission like *Rickettsia bellii* maintained in Ixodes loricatus [64]. Studies on infectious agents of O. megnini are few and mostly carried out retrospectively. First, as early as 1948, infection of *Coxiella* burnetii, the causative agent of Q fever, was recovered from O. megnini collected from dairy cattle in Southern California [24]. Q fever, first reported in Australia as an outbreak in 1935 in nine patients [65], has now been listed as a zoonotic disease transmitted to humans primarily through inhalation of contaminated dust or aerosols and through ticks. Infection with C. burnetii is therefore recognized as an occupational hazard for people who work with or around waste and birth products of livestock and may include farmers, veterinarians, zoo, and slaughterhouse workers [66]. Although ticks may readily transmit C. burnetii in experimental systems, they only occasionally transmit the pathogen in the field. Furthermore, there are many Coxiella-like bacterial endosymbionts which are widespread in ticks and may have been misidentified as C. burnetii. Desjardins and co-workers in [67] examined the presence of antibodies and DNA of *C. burnetii* in horses, ticks, and equine environment and the potential expression of clinical disease in horses in Southeast France, a region known to be hyperendemic for human Q fever [68–70]. Although few horses (4–12%) reported as seropositive and DNA in ticks and dust being qPCR positive, horse blood was qPCR negative and did not observe any statistical association between seropositive horses and positive ticks. Although the analysis consisted of 149 ticks, none of them were *O. megnini* [67].

One specimen of *O. megnini* collected from a human in Turkey was tested for Rickettsial DNA and reported PCR negative [16]. An early record from Mexico shows two cases of spotted fever *Rickettsia* infested with nymphs of *O. megnini* [23]. Studies have shown that *O. megnini* can be naturally infected with *Ehrlichia canis* but does not transmit the agent [71]. Experimental transmission of *E. canis* by laboratory-reared *O. megnini* was attempted, but neither transstadial nor transovarial transmission occurred [71]. A specimen recovered from a child who had serologic evidence ehrlichiosis was examined microscopically, but no evidence of infection was found [71]. In a study carried out to determine equine Lyme borreliosis in a large horse riding school in Natal Province, South Africa reported *Borrelia burgdorferi* seropositive cases of 3 horse riders and owner of a stable, 71 horses, and 5 dogs, but none of the *O. megnini* specimens collected from these hosts were positive for the infection [72].

A laboratory study carried out on *O. megnini* collected from the ear canal of 11 race horses in Sri Lanka investigated the presence of three infectious agents: *Rickettsia*, *Theileria*, and *Babesia* and reported that the ticks collected from two horses being PCR positive for *Babesia* infections [25]. However, there are no records whether these horses showed any clinical symptoms of babesiosis. The study provides the first record of *Babesia* infections in *O. megnini*. Further investigations to confirm the *Babesia* species and blood samples from horses to verify its vector capacity are important. In 1967 Uilenberg reported that the vector of equine piroplasmosis is unknown and reported the presence *O. megnini* horses together with other tick species [73].

#### 6. Human infestations

Presence of ticks in the human external auditory canal is a common parasitic otopathy reported in many parts of the world including South Africa [4, 74], Chile [3], the USA [75], Nepal [76], Malaysia [77], India [78], and Sri Lanka [79].

However, only few cases are presented with O. megnini, most of these are either associated with horse riding or grooming or farmers working closely with livestock [3, 7, 26, 71, 74]. The first record is in as early as 1917 [26]. Seven otoacariasis cases of O. megnini infesting human ear canal have been reported in New Mexico, USA [75]. More recent cases came from South Africa where a 15-year-old girl from Pretoria, a keen equestrian visited a riding school east of Pretoria and acquired infestation possibly while she was grooming or riding her horse [4]. Another case of 13-year-old girl reported with O. megnini inside her ear canal after a riding holiday in the Eastern Cape, South Africa [74]. Five patients visiting the Ear, Nose, Throat (ENT) clinic in Nuwara Eliya General Hospital in Sri Lanka reported having O. megnini in the ear canal. All these patients are horse riders or stable worker from a racecourse nearby [7]. In Turkey, O. megnini infections were reported from 29 females and 2 males between the ages from 17 to 72 years involved in agriculture and livestock mostly living in rural area but with no complications [80]. This tick was found in the ear of a woman who had the habit of basking in the sun on the lawn near a sheep shed at the Sheep Breeding Research Station in Sandynallah, India [18].

Tick paralysis is the most widespread and dominant form of tick toxicosis. Usually, the intra-aural tick infestation results facial paralysis, edema [81], otitis externa, bleeding [82], and acute labyrinthitis [83]. Human ear infestations by O. megnini, however, do not result in paralysis although irritation and pain was common [75]. However, there is one human case from 1958 reporting tick paralysis following infestation of *O. megnini*. Since having *O. megnini* inside the ear canal can be very painful in humans, development of the larva to next stage nymph is unlikely because the patient in this case becomes aware of the tick after the larva has engorged [84]. For O. megnini infestations, differential diagnosis should be followed whenever painful otitis externa with wax and debris is not responsive to conventional treatment [84]. Since the tick feeds intermittently and does not attach firmly, it may be easier to flush it from the ear canal unlike the hard ticks [84]. Tick inside the ear canal is removed using various methods. K.V.G Medical College, India recommends the use of turpentine and xyclocane prior to removing the tick [78]. However, the ENT clinics in general hospitals in Kandy and Anuradhapura in Sri Lanka use two different methods to remove the tick inside the ear canal [85]. In Anuradhapura General Hospital, lignocaine is used as a local anesthetic and the tick is removed immediately after using a suction pump. In Kandy General Hospital, glycerine is used to fill up the ear canal and followed by removal of the tick 2–3 weeks later by using a suction pump [85]. The method using local anesthesia is best for removal of O. megnini as the tick does not attach firmly and permanently but feeds intermittently.

In addition to the ear, the *O. megnini* has been found attached to other parts of the body. Larval *O. megnini* in the conjunctiva of a 2-year-old child's eye has been reported from Arizona, USA [2].

#### 7. Control methods

Ticks can be controlled using acaricidal chemicals, natural repellants, and biological control agents. Application of synthetic acaricides: carbamate, organo-phosphate, synthetic pyrethroid, formamidine, macrocyclic lactone, and pyrazole have played pivotal role in controlling both soft and hard ticks in the world [86]. Combinations of hexachlorocyclohexane, xylol, and pine oil provide protection from *O. megnini* for a minimum of 17 days [87]. Using insecticide impregnated ear tags are shown to be effective [88]. A list of acaricide recommended for tick control including *O. megnini* is given in Spackman and Lloyd [89]. Feeding sulfur to calves

does not have any effect on controlling *O. megnini* in the ear canal of the host [90]. Moreover, ivermectin is effective for controlling arthropod pests of livestock, but it is not an effective control measure for nymphs of O. megnini in the ears of cattle and horses [61]. However, control of O. megnini is challenging, due to its nidicolous lifestyle, abundant progeny and site of attachment deep in the ear canal [41]. Although acaricides are the best control and eradication effort because they offer quick and cost-effective suppression of tick populations, long-term use has developed acaricide resistance in many tick species worldwide and thereby reducing their effectiveness in controlling ticks [91, 92], impaired environmental and human health with negative effects on non-target organisms, and poor quality in animal products (e.g., milk, meat, and hide; [93]). Regular monitoring of the ticks for development of resistance against the acaricides used is therefore important. Detection of resistance level of an acaricide in a tick population is important before applying it as a control measure. Susceptibility of larvae of O. megnini to four acaricides, Permethrin, DDT, Malathion, and Flumethrin, has been tested in an O. megnini population in the stabled horses in Nuwara Eliya racecourse in Sri Lanka [94]. Flumethrin is the most susceptible acaricide against O. megnini, while the presence of resistance for DDT and possible presence of resistance to other three acaricides tested have been reported. Prevalence of the mutations in the resistant gene/genes has to be investigated to conclude the extent of resistance in O. megnini for these chemicals.

Use of alternative and more sustainable control measures as biological control and host immunization are therefore increasing rapidly [95], and the application of acaricide substitutes such as the extracts of plants like *Azadirachta indica*, *Calotropis procera*, and *Nicotiana tabacum* [96] is also being promoted. Although plant extractions have been used in general tick control, there are no studies conducted specifically for *O. megnini*.

In the biological control of ticks, Samish and Rehacek [55] have listed three types of potential natural enemies including pathogens like bacteria, fungi, and nematodes that infect ticks, predators like birds and ants, and parasitoid dipterans and hymenopterans that deposit eggs on ticks. Later, Samish et al. [95] have shown that these natural enemies can be used as potential candidates in controlling some hard and soft tick species under field and laboratory conditions. Bacterial species such as Rickettsia sp., Cedecea lapagei sp., and Proteus mirabilis, which are pathogenic to Dermacentor andersoni, Amblyomma hebraeum, and Hyalomma marginatum [97] may change the tick behavior, interfere with the development, cause changes in salivary and ovarian tissues, and also induce abnormalities in subsequent generations. Among protozoans, Nosema ixodis and Babesia bigemina cause deaths and minimize egg production of Rhipicephalus microplus, respectively [98]. Six out of 57 major genera of entomopathogenic fungi are known to infect ticks [99]. Of these fungi, *Metarhizium anisopliae* and *Beauveria bassiana* are shown to be effective in controlling *R. microplus* and *Rhipicephalus appendiculatus* [55, 100]. Even though nematodes have been listed as potential biological agents against ticks, these pathogens have never been reported in ticks in nature. However, under laboratory conditions some nematodes infest Rhipicephalus annulatus [55].

The role of predators in controlling ticks has been well documented. So far, predator-tick relationship of 28 arthropod families has been recognized of which many are ants (*Hymenoptera: Formicidae*), followed by carabid beetles (*Coleoptera: Carabidae*) and some spiders (*Araneae: Lycosidae*; [101]). Other than arthropods, some vertebrates like the amphibians (*Bufo parcnemis*; [55]), birds (oxpeckers, egrets, domestic fowl; [95]), and mammals (shrews, rodents; [55]) occasionally feed on ticks. Among the ants, 27 species belonging to 16 genera including *Solenopsis* sp., *Pogonomyrmex* sp., *Iridomyrmex* sp., *Aphaenogaster* sp., and *Monomorium* sp. have been identified as potential biological control agents of ticks [55, 95]. They target different developing

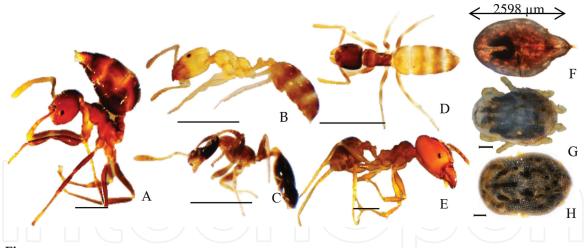
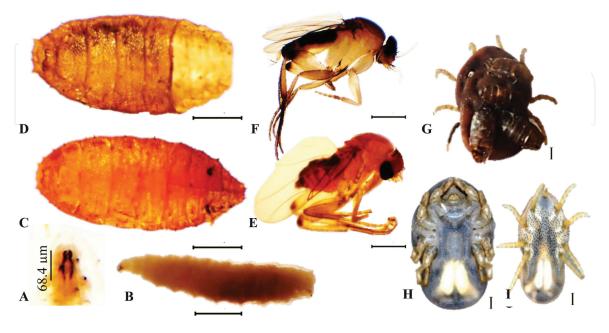


Figure 5.

Ant species that infest different life-history stages of Otobius megnini. (A) Crematogaster sp., (B) Monomorium sp. 1, (C) Monomorium sp. 2, (D) Tapinoma melanocephalum, (E) Pheidole sp., (F) engorged larvae, (G) nymph, and (H) adult of Otobius megnini. Scale bar represents 1 mm.

stages of the ticks. However, many of these ants occasionally target ticks as their main food source but are natural predators of tick species including *Argas miniatus* [102], *R. microplus*, and *R. annulatus* [102], *O. megnini*, and *Ornithodorus moubata* [53]. Five ant species, *Tapinoma melanocephalum*, two species of *Monomorium*, one species of *Pheidole*, and one species of *Crematogaster* feed on eggs fed and unfed larvae and adults of *O. megnini* (**Figure 5**) [103]. Among these, *T. melanocephalum* is the best predator as it feeds all free-living stages (eggs and adults) [103].

Among the opportunistic parasitoid dipterans, *Megaselia scalaris* and *Megaselia rufipes* (Family: Phoridae) have been identified infesting hard and soft ticks successfully [55, 95, 104]. *Megaselia scalaris* actively infests laboratory colonies of *O. megnini* [104] and other tick species [105]. It is a cosmopolitan fly, 2–3 mm long with medical, forensic, and veterinary importance commonly known as scuttle flies or hump-backed flies due to their erratic movement on surfaces and morphological features of the thorax, respectively (**Figure 6**) [104, 106]. These flies are capable



#### Figure 6.

Life-history stages of Megaselia scalaris and Otobius megnini. (A) Cephalopharyngeal structure of second instar larva, (B) second instar larvae, (C) pupa, (D) open pupal case, (E) female imago, and (F) male imago of M. scalaris. (G) O. megnini adult female with several pupa attached on dorsal side. (H) Ventral side of a healthy O. megnini female. (I) O. megnini nymph. Scale bars represent length of 1 mm.

of exploiting diverse ecological niches in tropics and subtropics [107]. *Megaselia scalaris* has adapted to polyphagous lifestyle, feeding, and breeding in wider spectrum of plant and animal matter [107–109]. They are attracted to putrid odors and lay eggs on decaying organic matter. The larva (maggot) undergoes two molts leading to three larval stages. Saprophagous (feeding on decaying organic matter), sarcophagous (feeding on flesh), and necrophagous (feeding on carrion) modes of feeding, as well as parasitic behaviors of *M. scalaris* larvae are well documented [107, 110]. Larvae of *M. scalaris* feed on larvae and nymphs of *O. megnini*, and when the development of the fly is completed, pupae attach to adult ticks, and all nymphs were found dead [103].

## 8. Conclusions

Infestation of *O. megnini* has become a problem worldwide. Controlling tick populations is hard because of its life cycle and seasonal dynamics and development of acaricide resistance. Among horses, *O. megnini* infests only well-groomed horses but not those with hairy ears. If the horses are left without trimming the hair in and around ears during racing off season, together with integrated pest control methods, the infestations can be effectively controlled and will alleviate the painful experience and other complications in the horse having the ticks inside the ear. The presence of many infectious agents has been detected in the tick; however, whether *O. megnini* acts as a vector or a reservoir in spreading the infection needing to be substantiated.

### **Conflict of interest**

None.

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