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Case Report

Severe Skin Lesions Caused by Persistent Bites of the Stable Fly *Stomoxys calcitrans* (Diptera: Muscidae) in a Donkey Sanctuary of Western Spain

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ABSTRACT

Animal sanctuaries are important organisations that promote animal welfare and health as well as social awareness. Following the appearance of several donkeys with severe and distinctive skin lesions in a sanctuary from western Spain in 2021, a multicomponent study was performed to rule out the possible causes. The lesions were mainly concentrated on the extremities and, to a lesser extent, on the face and/or chest. The use of cotton leggings on their extremities as an external barrier to prevent them from bites and treatments with antiparasitic, antiseptic washes and dermatitis lotion showed to be effective measures to improve the donkey lesions. Skin scraping was negative for any relevant causative agents. Histopathological examination of the lesions showed an inflammatory infiltrate at the superficial dermal level, compatible with diffuse chronic dermatitis. A field entomological study was conducted from May to October 2021 to ascertain if any Diptera was responsible for these lesions. Considerable numbers of the stable fly *Stomoxys calcitrans* were recorded in both sticky traps and decomposing straw bedding. Several species of hematophagous Diptera were also recorded in lower numbers in other traps placed on the farm facilities. According to the entomological data, the location of the bites and clinical signs, it was concluded that *S. calcitrans* was the main cause of the skin lesions in the donkeys. To our best knowledge, this is the first clinical case of donkeys affected by the stable fly in Spain. For differential diagnosis, a brief discussion of the evidence caused by other biting Diptera groups is provided.

1. Introduction

Many of the dermatological diseases in equids have similar clinical features [1], so in most cases they remain without reaching an assertive diagnosis, constituting a challenge for clinical veterinarians [2,3]. In the economic aspect, dermatopathologies in equids cause high losses in the equine industry due to decreased performance, aesthetic alterations, high treatment costs, and eventual deaths or discards [3,4].

A large percentage of crawling and flying ectoparasites such as mites, lice, ticks, and insects might affect the skin surface of horses [4,5] and other animals by their annoying direct bites, feeding on secretions and wounds, causing myiasis, transmitting infectious agents, inducing hypersensitivity and toxic reactions and even causing death if exposure is overwhelming [6,7]. Lack of ento-

Abbreviations: CDC, Center Disease Control; WOT, Wind Orientated Traps; BG, Biogents.

Conflict of interest statement: The authors declare no conflicts of interest.

Animal welfare/ethical statement: Donkeys belong to The Donkey Sanctuary "El Refugio del Burrito" and data were obtained when animals were subjected to regular veterinary checks by official veterinarians. The study was performed under the guidelines of The Donkey Sanctuary ethical committee: https://www.thedonkeysanctuary.org.uk/what-we-do/knowledge-and-advice/research/our-research-aims.

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mological knowledge may prevent an accurate assertive diagnosis, which would not allow the implementation of appropriate preventive measures.

Among the most important blood-feeding flies are the members of the family Muscidae, including the horn fly *Haematobia irritans* and the stable fly *Stomoxys calcitrans*. Both are considered significant global economic pests that affect mostly confined and free-roaming livestock [8,9]. Their direct effects on hosts cause disturbance, skin lesions, reduced feed intake, stress and blood loss, but they can also act as mechanical vectors (rarely as biological vectors) of several bacterial, viral, and protozoan pathogens [10]. Other tiny biting flies are also associated with episodes of disturbance or irritation to grazing animals. Members of the family Simuliidae, also known as black flies, cause painful dermal trauma that leaves a bleeding and extremely painful cut that can become infected. Animals may suffer from simuliotoxicosis characterized by severe dermatitis, associated with exudation, encrustation and thickening of the epidermis, occasionally resulting in death from toxemia, shock, and exsanguination [11,12]. Similarly, biting midges of the family Ceratopogonidae, mostly *Culicoides* species, but also *Leptoconops,* can cause economic losses and health problems in animals and humans [13]. Particularly *Culicoides* midges cause a hypersensitivity reaction leading to a recurrent seasonal allergic skin disease known as “sweet itch” that affects mostly horses, but also donkeys, goats, and sheep [14–16]. Other hematophagous Diptera are also ferocious biters (i.e., Tabanidae and Hippoboscidae) but rarely cause lesions like those described above [5].

Therefore, understanding which insect species is responsible for the damage will help in the early treatment of the animals and promote adequate control strategies to prevent and retain the spread of equine and livestock diseases. A series of clinical and pathological tests together with an entomological field study were carried out to elucidate the causative agent of the skin lesions observed in donkeys of The Donkey Sanctuary in western Spain.

2. Case Description

2.1. Study Area

The study was conducted in The Donkey Sanctuary “El Refugio del Burrito”, located in a rural area of Badajoz (Extremadura, western Spain, 38°09′27.7″ N, 6°34′28.8″ W, 615 m a.s.l.). The studied farm covers an area of ca. 37 hectares of pasture with approximately 2,000 olive trees and 120 holm oaks.

2.2. Animal and Farm Characteristics

There were approximately 154 animals (donkeys, mules, and ponies) of mixed breeds in the sanctuary facilities, divided into different sections. Each of the seven large barns located in the center of the farm host 5 to 25 animals. Barns (30 m long × 17 m wide × 5.5 m high) are constructed of a metal frame with internal and external concrete walls; above the internal walls opaque plastic box sheeting was utilized to create natural light within the barn and the roof is insulated with metal sheeting and the doors are constructed of a metal frame with wood and glass infill. The barn is split into three sections: a central bedded area (straw or shaving bedding) on a concrete floor and two feed passageways on either side of the central bedded area. Complete bedding replacement was done every 15–20 days depending on the season. The animals spent most of the day outdoors in extensive pastures with olive trees that provide shade and shelter. They have free access to their barns at all times. The diet consisted of straw, hay and free access to pasture. Vitamin and mineral supplements were also provided during the winter period. Manure was stored in a large open-air heap at the farm corner and removed 2–3 times a year.

2.3. Clinical Study

The donkeys that showed the most evident clinical signs of hypersensitivity (n = 7) were selected to be examined and treated monthly from May 2021 to February 2022. The donkeys varied in coat color (one grey/black, four grey/brownish, one steel grey and one brown/chestnut), sex (three females and four castrated males) and age (between 6 and 28 years). The location of the skin wounds was noted and assigned on a scale from 0 (without lesions) to 5 (severe lesions).

The clinical signs of the skin lesions started as little pruritic papules on the skin accompanied by excoriations (Fig. 1A) and progressed to hemorrhagic crust formation, hair breakage or patchy hair loss (alopecia) (Fig. 1B), and scaly or oozing lesions. Subsequently, chronic cases result in skin lichenification, nodule formation or thickening, leaving a relatively large lesion with or without recovering hair (Fig. 1C, D). This dermatitis is usually accompanied by marked pruritus, resulting in lesions associated with self-induced trauma. The lesions were concentrated primarily on the extremities (85% of all individuals) and, to a lesser extent, on the face and/or chest (25%). At the beginning (May 2021), the average score of the lesions of the seven donkeys was recorded as 3.0 (lesion score ranged from 1 to 5), while at the end (February 2022) it decreased to 0.7 (lesion score ranged from 0 to 1). Skin scrapings (n = 3) were collected and sent to an external laboratory (Gasset Laboratories, Granada, Spain) to rule out possible scabies, bacterial, and/or fungal involvement. All samples resulted negative to any relevant causative agents. A biopsy of the chest of one of the affected animals was analyzed (Gasset Laboratories, Granada, Spain). Histopathology of the lesion showed a superficial dermal inflammatory infiltrate that could be compatible with chronic diffuse dermatitis. In May, the donkeys were initially treated with the topical ivermectin antiparasitic mixed with vaseline used as a repellent (Knottenbelt, personal communication), and corticoids (Diprogenta, Ulfaprisol, and/or Equisolon) applied together with insect repellent (Protec Plus). From June to November, skin wounds were washed with both antiseptic (chlorhexidine) and natural lotion for dermatitis (Derfen). Cotton leggings were fitted in the worst affected animals as a physical barrier to protect their extremities from fly bites (Fig. 2A).

2.4. Entomological Study

A multi-sampling approach was conducted to collect arthropod families of major interest either as nuisance flies or as blood-sucking insects with the capacity to produce wounds on the skin of domestic animals. Suspecting the involvement of hematophagous flies such as *S. calcitrans*, homemade white plastic rectangular cardboards (30 × 25 cm) coated with glue were placed. These traps are commonly and effectively used to assess the population dynamics of this fly species in the UK [17]. In total, ten sticky traps were deployed in warehouses and animal passage areas (fences and walls) during two periods: at the beginning of summer (11–21 June 2021) and late summer (12–22 August 2021).

Three CDC-Miniature Light traps (model 1212, John Hock, USA), two BG-Sentinel traps (Biogents, Germany), and two homemade WOTs (wind-oriented traps), were set for 24 hours (16–17 September 2021) at different outdoor locations on the farm facilities to assess the occurrence of diverse flying insects. CDC-Miniature Light traps and BG-Sentinel traps baited with attractants (CO_2 and BG-lure, respectively) were used to capture mosquitoes, *Culicoides* and sandflies, among other insects [18]. Wind-oriented traps baited with pig liver and blood were primarily used to collect members of the Calliphoridae, Muscidae, and Sarcophagidae families [19,20]. Sweep netting was occasionally used on the donkeys to capture dipterans perched on them.

Fig. 1. Some examples of donkeys showing different lesions in their lower extremities. (A) Little pruritic papules on the skin accompanied by excoriations; (B) Formation of hemorrhagic crust; (C and D) Chronic skin lesions with lichenification of skin and hair loss.

At the same time as the placement of the traps, two samples (ca. 3 kg of fresh weight) of soiled bedding containing a blend of straw, fecal material and urine (10–12 days maturation) (Fig. 2B) were collected. Samples were placed in trays inside entomological boxes (BioQuip, USA) at a temperature of 22–24°C, relative humidity 70% and a controlled light period of 12/12 hours for 5 weeks in the laboratory until the last specimens emerged (Fig. 2C). Insects were retrieved every 3 days by mechanical aspiration.

Insects collected by the previously described methods were identified according to various determination keys [21–24]. Due to the large number of *S. calcitrans* flies glued on the sticky traps, the densities of these flies were estimated based on a routine grid calculation. High numbers of *S. calcitrans* were recorded on the sticky traps (Fig. 2D), accounting for up to 9,456 specimens (Mean ± SD = 436.6 ± 160.9 specimens/sticky trap in the first period and 277.7 ± 177.4 specimens/sticky trap in the second period). Approximately 97% of the total flies glued on sticky traps (Fig. 2E) corresponded to *S. calcitrans*, which are characterized by their long proboscis protruding from the front of the head (Fig. 2F). Species identification (n = 2) was molecularly confirmed by amplification of the cytochrome oxidase 1 (cox1) gene using the primer set LCO1490 and HCO2198 following PCR protocol as described previously by Folmer et al. [25]. The identity showed 100% homology with public sequences of *S. calcitrans* from GenBank (accession numbers: KU932147.1 or KM571650.1 from Poland and Canada, respectively). Other hematophagous species were also recorded, *Tabanus bovinus* s.l., (n = 2), *Ornithomya* sp. (n = 1), and *Hippobosca equina* (n = 1). Other specimens of Muscidae and Fanniidae (not counted) were recorded. The WOT, BG-Sentinel, and CDC traps collected a wide variety of dipteran species including hematophagous mosquitoes (two species, 13 specimens), sand flies (two species, 160 specimens), *Culicoides* midges (six species, 22 specimens), and *H. irritans* (a single specimen), but in relatively low numbers (Table 1). Typical members of the family Muscidae and Calliphoridae were also collected (Table 1). Sweep netting was a very inefficient collection method, only capturing a few *Musca* sp.

Large numbers of *S. calcitrans* emerged from the two bedding substrates (n = 319 and 317, Fig. 2C), with ca. 900 g each of dry organic material (weight after 5 weeks). Stable flies emerged con-

Fig. 2. Representation of some phases of the study; (A) a donkey fitted with cotton leggings, (B) bedding collection, (C) emergency entomological box with bedding, (D) sticky trap with high numbers of *Stomoxys calcitrans* (E) glued specimen of *S. calcitrans,* and (F) detail of the long, stout and black proboscis of *S. calcitrans*.

Table 1
Counts of adult flying diptera with medical and veterinary importance collected by the multi-trapping approach on 16–17 September 2021 in The Donkey Sanctuary from western Spain.

Trap	Family	Species	nº
BG-Sentinel	Culicidae	*Culex pipiens* s.l.	7
	Culicidae	*Culiseta longiareolata*	4
	Muscidae	*Stomoxys calcitrans*	6
	Psychodidae	*Phlebotomus perniciosus*	25
	Psychodidae	*Sergentomyia minuta*	1
WOT	Calliphoridae	*Calliphora vomitoria*	8
	Calliphoridae	*Lucilia* sp.	23
	Sarcophagidae	*Sarcophaga* sp.	6
CDC	Ceratopogonidae	*Culicoides circumscriptus*	11
	Ceratopogonidae	*Culicoides imicola*	5
	Ceratopogonidae	*Culicoides newsteadi*	2
	Ceratopogonidae	*Culicoides fagineus*	1
	Ceratopogonidae	*Culicoides paradoxalis*	1
	Ceratopogonidae	*Culicoides alazanicus*	1
	Ceratopogonidae	*Culicoides* sp.	1
	Muscidae	*Haematobia irritans*	1
	Muscidae	*Musca autumnalis*	2
	Psychodidae	*Phlebotomus perniciosus*	133
	Psychodidae	*Sergentomyia minuta*	1

tinuously from the boxes throughout the entire period (42.7%, 1st week; 22.6%, 2nd week, 20.9%, 3rd week; 10.3%, 4th week, and 3.5%, 5th week). *Musca domestica* (n = 34) and *Musca autumnalis* (n = 1) were also recorded together with other nonbiting flies associated with manure (Sphaeroceridae, n = 235) and straw piles (*Drosophila repleta*, n = 3).

3. Discussion

Given the abovementioned results, it is reasonable to attribute the lesions caused in donkeys exclusively to the biting activity of the stable fly *S. calcitrans*. These flies feed mainly on the lower part of animals, mostly on legs and lower limbs, but also on the ventral abdomen, chest and back, especially in cattle and equines [26], which is consistent with our findings. The persistent biting activity of *S. calcitrans* might also affect other animal species (i.e., dogs) causing "fly-strike dermatitis" with clinical lesions typically seen in other body locations (i.e., folding edges and/or tips of the ears and muzzle) [27]. The stable flies possess a telmophagous biting apparatus, so their bites cause multiple breaches in the skin tissue that form a micro-hematoma, which after feeding leaves a small drop of blood. In addition, bites are often placed in a defined pattern of three or four spots in a chain or group [5]. However, other pool feeders produce similar lesions but can be separated by the following key aspects. (1) *Culicoides* biting midges typically produce a seasonally recurrent highly debilitating allergic dermatitis accompanied by intense pruritus mainly affecting the mane and tail area but also the back line [28]. Despite wounds being similar in appearance at the start of the process, neither the location nor the species implicated match our data. (2) There is little evidence of sand flies attacking equids except by swarm attacks, bites typically occur all over the body, and the legs tend to be less affected [5]. (3) *Haematobia irritans* aggregates to bite around the back and shoulders, less frequently on the neck and withers, and on the legs and belly during the hottest part of the day [29,30]; (4) Tabanid bites produce a marked painful, pruritic skin response, showing weals with a central hemorrhagic mark; however, they are massive in size and easily observed apart from being present in highly specific breeding habitats requiring aquatic and semiaquatic sites in bushes and forest habitats [31], which were not present in the study area. (5) Simulids cause extreme irritation an inflict painful bites, mainly on the inside of the ears and belly, and produce an insect bite hypersensitivity (simuliotoxicosis) with different pattern than *Culicoides* [32]. In addition, they specifically breed in running watercourses [33], which were not seen in the vicinity of the study area, and (6) loose flies (typically *Hippobosca*) congregate around the perineum and inguinal regions, causing significant lesions with

persistent blood loss. They are not easily disturbed and cause local irritation and fly-worry [34].

Identification of *S. calcitrans* adults is straightforward, as this species has distinctive long, bayonet-shaped mouthparts called proboscis, which makes it easily distinguishable from any other ordinary fly species. In Spain, *Stomoxys* can only be misidentified with *Haematobosca*, but both genera can be separated based on the shape and length of the palpi relative to the proboscis [35]. *Stomoxys calcitrans* is a cosmopolitan zoophilic species, whose painful bites affect animal welfare, causing weakness, stress, anemia, secondary infections, and reducing grazing [9]. In Spain, it is well-distributed throughout most of the territory [35]; however, no detailed studies have been published, except for some on the prevalence of stable flies in livestock areas [36]. Locally, *S. calcitrans* has been recorded as a possible vector of *Trypanosoma evansi* in camels from the Canary Islands [37].

The developmental sites of *S. calcitrans* are diverse and usually associated with decaying or rotting vegetable matter such as organic waste of plant origins, decaying hay, alfalfa, silage, straw, sugarcane, beached seagrass, lawn cuttings and waste horticultural products, but immatures can also develop in fresh cow manure, old manure, compost, animal waste such as poultry litter and bedding contaminated with urine and animal excrements [38–41], which is consistent with our findings. The massive emergence of nearly 650 specimens from 3 kg of organic bedding, gives an idea of the magnitude of the problem considering the large area covered by soiled bedding in the sanctuary.

Control of *S. calcitrans* is very complex and should include a combination of multiple measures such as adult trapping, sanitation, drugs/products, bedding management, biological control, physical barriers and animal protection, among others [4,41]. In our study, we observed that open bleeding crusty lesions experienced a good recovery over the first weeks; however, donkeys with lichenification of skin did not recover hair and those remained as chronic skin lesions. This improvement might be attributed to the application of prophylaxis, use of “leggings” and the reduction of adult *S. calcitrans* numbers through mass trapping as demonstrated by Mottet et al. [42]. However, another possible explanation that cannot be excluded is that *S. calcitrans* populations decline naturally at the end of the warm season, as this species is recorded as being highly seasonal in Europe [17].

Ectoparasite infestations are a growing concern for both owners and equine veterinarians, and better entomological knowledge can help to make decisions when faced with infestations. To the best of the authors’ knowledge, this is the first documented evidence of stable fly cutaneous damage in donkeys from Spain and reinforces that *S. calcitrans* can be an equine veterinary problem and should be considered as a causal agent of skin lesions.

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