https://doi.org/10.22319/rmcp.v14i2.5881

Review

Importance of *Haematobia irritans* in cattle in Mexico: Current situation and perspectives. Review

Roger Iván Rodríguez Vivas [a*]

Carlos Cruz Vázquez [b]

Consuelo Almazán [c]

Juan José Zárate Ramos [d]

[a] Universidad Autónoma de Yucatán. Facultad de Medicina Veterinaria y Zootecnia. Carretera Mérida-Xmatkuil Km 15.5, Mérida, Yucatán, México.

[b] Tecnológico Nacional de México. Instituto Tecnológico El Llano, Aguascalientes, México.

[c] Laboratorio de Inmunología y Vacunas; Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Querétaro, México.

[d] Universidad Autónoma de Nuevo León. Facultad de Medicina Veterinaria y Zootecnia, Campus de Ciencias Agropecuarias. General Escobedo, Nuevo León, México.

*Corresponding author: rvivas@correo.uady.mx

Abstract:

The horn fly *Haematobia irritans* is a cosmopolitan hematophagous ectoparasite of great importance in livestock. In Mexico, *H. irritans* is distributed across the country, and is found during the whole year. The fluctuation of *H. irritans* population is related with climate conditions. Despite its wide distribution, the effects on animal health, and its negative impact on meat and milk production, little data exists on its infestation and epidemiology is limited. This paper is a review on the current situation of *H. irritans* in cattle in Mexico, its economic impact, control methods, perspectives, and research opportunities.

Keywords: *Haematobia irritans*, Horn fly, Epizootiology, Control.

Received: 25/11/2020

Accepted: 15/09/2022

Introduction

Horn fly *Haematobia irritans* (Linneaus, 1758) is a dipteran belonging to the Muscidae family. This fly is a widely distributed hematophagous ectoparasite of cattle, that negatively impacts beef and dairy production[(1)]. The direct effects of *H. irritans* parasitism include blood loss and skin damage, as well as constant restlessness of infested animals, which cause reduction in production of meat and milk[(2)]. The impact of *H. irritans* on animal production is related to infestation levels, which depends of animal characteristics and regional environmental conditions[(3)]. In Mexico, *H. irritans* is geographically distributed in the country during the whole year[(2)].

Control of *H. irritans* infestations is mainly attempted by use of insecticides from chemical families such as pyrethroids, organophosphates, phenylpyrazolones, growth regulators, and insect growth inhibitors, among others. However, over frequent and incorrect use of these insecticides has led to the selection of insecticide-resistant *H. irritans* populations. Currently, resistance to pyrethroids and organophosphates in *H. irritans* is known[(2)].

Environmentally sustainable control strategies include cultural management of manure and biological control measures, such as the use of natural enemies, entomopathogenic agents and botanically-sourced repellents and pesticides. Immunological control by vaccination can prevent or reduce the insect hematophagy, but experimental results of this method are still preliminary and vaccines against fly infestation do not exist[(4)]. Integrated pest control (IPC), the combined and rational application of existing methods, is the most effective method of horn fly reduction[(2)]

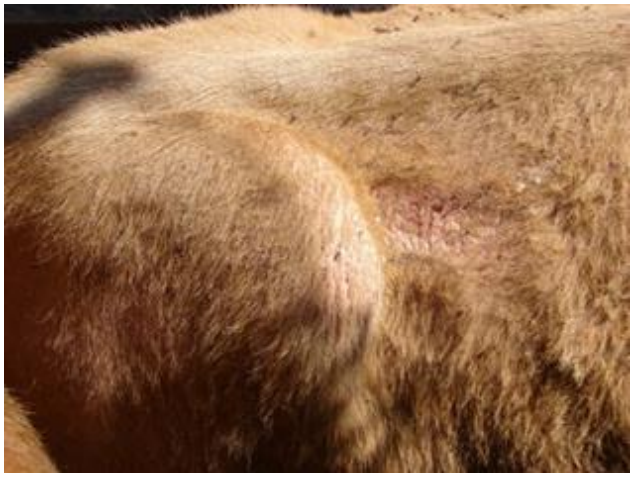
This paper presents a review on the current situation of *H. irritans* in Mexico, the economic impact in cattle, the available control methods, and perspectives and research opportunities.

Direct and indirect effects of *H. irritans* on cattle

Direct damage. Female and male *H. irritans* feed from 20 to 38 times a day, consuming small portions of blood in each feeding, with an average of 10 µl per day per fly[(5)]. By

piercing the host's skin, this hematophagous action produces damage and a reduction in skin quality[3]. Skin damage includes blackheads and orifices, in which most damage is apparently due to dermal inflammatory responses (Figure 1). Eosinophilic infiltration, eosinophilic folliculitis, and furunculosis with alopecia can occur at the feeding site[6].

Figure 1: Severe skin damage, including alopecia and hypercheratosis, in a cow without ectoparasite treatment during the season of highest intensity of *Haematobia irritans* in the northern Gulf of Mexico region



Source: C. Almazán

Disease Transmission. *Haematobia irritans* is the intermediate host of *Stephanofilaria stilesi*, a nematode that causes skin lesions in cattle and is reported in cattle in Canada and the western and southwestern United States of America (USA). This fly can also mechanically transmit several species of *Staphylococcus* bacteria, which can cause mastitis in dairy cows[7]. In addition, it is involved in the mechanical transmission of other pathogens such as *Trypanosoma vivax* and *T. evansi*, *Francisella tularensis*, *Corynebacterium pseudotuberculosis*, *Parabronema skrjabini* and *Anaplasma marginale*[8-10].

Economic impact

The weight loss due to *H. irritans* infestations in beef cattle has been estimated in 3.25 kg/cow in Brazil in average per year[11], and 0.028 kg/cow/d (305 d of lactation, 8.54 kg per cow) in Argentina[12]. In a study done in the USA, heifers treated against horn fly exhibited 14 % more weight gain than untreated control[13]. Control measures also benefited cows, which won 14.4 kg more after treatment[14].

Milk production also drops due to *H. irritans* infestation, with reductions of approximately 27 kg of milk/cow/yr have been reported on dairy farms in the US[15]. Indeed, in 2016 an estimation of US 1.75 billion dollars due to the direct effects of *H. irritans* on dairy cows was reported. This parasite also generates the additional expense of US 60 million dollars annually for chemical control[16]. Based on Mexico's potential at risk cattle population, estimated annual losses attributed to *H. irritans* amount to US$ 231.66 million[1]. However, evaluations have not been done of losses generated through reduced pregnancy rates, transmitted pathogens and the need for additional control measures.

Life cycle of *H. irritans*

In this review, the horn fly is referred to as *H. irritans*. However, it has been suggested that there are actually two morphologically similar subspecies of horn fly, *H. irritans irritans* and *H. irritans exigua* (buffalo fly). The former is distributed in Europe and America, and the latter in Asia and Australia[17].

Tropical and subtropical climates with average temperatures of 20 to 30 °C and relative humidity from 65 to 90 % are extremely propitious for development of *H. irritans*[17]. In Mexico, *H. irritans* is also distributed in temperate climates[18].

The adult *H. irritans* flies are 3 to 4 mm long, gray in color with dark stripes on the thorax, and with a pair of dark reddish compound eyes. It exhibits sexual dimorphism; the eyes are more separated and smaller in females than in males, and males have a slightly folded abdomen. On the host animal, this fly normally perches facing the ground[17].

The *H. irritans* host range is ample and its main host is cattle, although it also parasitizes sheep, horses, canines, water buffalo, bison and humans[19]. Animal color influences fly preference, with black animals attracting greater numbers of flies[2]. In cattle, bulls prove more attractive to *H. irritans* than steers or cows. The fly spends most of its life on the host, mostly feeding but also reproducing. Females may leave their host to lay eggs in extremely fresh manure; in fact, for *H. irritans* fecal attraction begins to disappear about 10 min post-defecation. To lay eggs, females spend postrated on fresh fecesone to ten minutes on feces[2]. Fly distribution on a host changes during the day. In the early daylight hours they tend to concentrate on the shoulders and back, then move to the abdomen midline and the sides in the afternoon, returning to the shoulder and back area at night[2]. Average adult lifespan is six to eight weeks. Longevity is inversely related to low temperatures, which negatively influence ovary development, mating, larval development, and adult emergence[17]. After

emergence, adults require three days for their reproductive organs to fully mature. Adults mate three to five days after emergence and oviposition occurs three to eight days after[20].

In the Americas, air temperature is the main climatic factor affecting the *H. irritans* life cycle and the humidity/temperature relationship is essential for fly reproduction[18]. Temperature influences infestation seasonality and is related to presentation of total or partial facultative diapause. In both, tropical and temperate climate regions, fly population decline during winter without entering into total diapause, while in temperate-cold climate regions diapause invariably occurs in winter[18]. Unsurprisingly, fly populations have been reported during the whole year in Mexico's humid tropics[21].

Regardless of the time of the day, *H. irritans* lays eggs in fresh feces, usually within the first two minutes after feces are excreted. One female can lay up to 400 eggs, which are deposited in groups of 20-25[22]. Eggs are oval-cylindrical in shape, slightly curved with a longitudinal medial groove, and yellow or white in color when laid, and become dark after. They range in size from 1.0 to 0.5 mm long by 0.34 to 0.39 mm wide[17]. To hatch, a temperature of 24 - 26 ºC and relative humidity near 100% is needed. Hatching normally occurs after a period of 20 to 48 h of incubation[20].

H. irritans larvae are yellowish-white in color, measuring 7 mm long. They present with a pair of posterior spiracles showing a "D" shape[20]. Larvae have three developmental stages (L1, L2 and L3). Development from L1 to L3 requires four to eight days, and pupation six to eight days. Both L2 and L3 larvae have anterior spiracles while L1 larvae lack them. The posterior spiracles allow differentiation between L2 and L3 stages: L2 larvae have two openings in the spiracles while L3 larvae have three. The larvae feed on bacteria in feces[23].

Development of pupa requires six to eight days[23]. The pupal stage is surrounded by the exoskeleton from L3, which darkens and hardens, forming a capsule called puparium[23]. Pupa development requires humidity and temperature conditions similar to those for larval development. After seven to eight days adults emerge and search immediately for a host to feed[20]. Diapause occurs at temperatures below 23 °C and pupae can survive prolonged periods of exposure to temperatures as low as -5 °C[22]. Under normal conditions, the life cycle is completed in 10 to 20 days[3].

Geographic distribution and population dynamics. In Mexico, *H. irritans* was reported for the first time in the state of Veracruz in 1984. It is currently known to be distributed throughout the country, mainly in association with livestock in extensive systems which facilitate its life cycle[2,24].

The population dynamics of *H. irritans* is related to regional climatic conditions, and flies are seen during the whole year in tropical climates. The intensity of *H. irritans* during the differs regionally, but always tends to show seasonality. Two population peaks can generally be observed between late spring and early autumn. In addition to regional climate, abundance can also respond to other environmental and management factors that may cause population fluctuations during a year and even between different years[25]. Populations do not develop significantly at altitudes higher than 1,800 m asl. In Mexico, the population dynamics of *H. irritans* generally exhibit a bimodal behavior, with a wide intra-annual fluctuations. Infestation seasonality is associated with temperature and relative humidity and the infestation index varies more in tropical than in temperate regions and decreases at high altitude[26,27,28].

The highest infestation rates of *H. irritans* are found from late spring to early autumn, and up to three population peaks occur in certain areas. During the summer months, infestation index values can exceed 4,000 flies per animal, while in less propitious periods it can drop to 200 to 450 flies per animal. Insecticide application and *H. irritans* resistance, as well as grazing and excreta management, may affect the index estimation in a herd[28]. In temperate climates, *H. irritans* population dynamics is bimodal and is considered seasonal, with increases from late spring to early autumn, and peak infestation rates in summer. Facultative diapause may occur during winter in temperate climates, therefore animal infestations are not observed[26,27].

Several generations of *H. irritans* may be produced in a year. In cold climates, 7 to 9 generations a year have been estimated, while in warm climates, the number of generations can range from 8 to 14[22]. in a semi-arid region of Brazil, thirty generations per year have been reported[20]. In Mexico, information on the number of generations produced by *H. irritans* per year does not exit. This information is essential to understand the parasite behavior and to elaborate control strategies.

Host resistance. *Bos indicus* breeds are less susceptible to ectoparasite infestation than *B. taurus* breeds[29]. Significant differences on *H. irritans* density between different *B. taurus* breeds have been observed. For example, the Chianina breed is more resistant to fly infestation than Angus, Hereford and Charolais breeds[30]. In Brazil, it was found that Guzerat x Holstein cross cattle had higher infestation levels than pure breed Guzerat cattle[31]. A study of infestation resistance done in southern Mexico reported fly counts on *B. indicus* animals to be equal or lower than on *B. taurus* animals[2]. Within the same herd, *H. irritans* infestation is not homogeneous, with more than 50% of a fly population parasitizing only 15-30% of the animals, which suggests that some animals are more susceptible to fly infestation[32]. Susceptibility to *H. irritans* infestations is influenced by animal color (dark-colored animals are more susceptible), size (large animals have higher levels), hair density, and sebum production (infestation is higher in animals with lower hair density and sebum production),

and hormones (higher testosterone levels favor higher infestation). Also, natural resistance, such as individual immune response and coagulation system, can influence infestation levels[33].

Estimating infestation as number of flies and the economic threshold (ET). Establishing the ET in *H. irritans* infestations requires estimation of the quantity of flies on the animals that would cause economic losses. Economic losses is understood as an amount of damage that would justify the cost of artificial pest control, while ET is the parasite population intensity that requires control measures to prevent losses that would exceed the cost of the control intervention[2]. Quantifying fly counts on animals is done using two methods: direct visual (DV) or indirect digital (IDV; i.e. photographs or video). In both methods, fly counts or images are obtained by trained persons at a distance of 1 to 4 m from an animal. Longer distances (5 - 10 m) can be used depending on animal docility[34-36], using binoculars[37]. In order to obtain the most accurate ET, counting should be done when flies are most visible on the animal and there is enough natural light. Accuracy may be lower during warmer time of the day since a high proportion of horn flies move to the lower abdomen. When ET is done on different days, it should be done at the same time, from 06:00 to 12:00 h[16,38,39]. In other reports, counts have been done from 15:30 to 19:00 h[36].

Whether with DV or IDV, counts must be done by trained personnel. Counts are normally done on one side of the animal and then multiplied by two to produce the total number of flies per animal, but counting can also done on both sides of an animal by two persons simultaneously[34-36]. Fly counts can be underestimated when fly density is extremely high. If fly density on the scapular, interscapular and costal regions is ≤25, they are counted individually but when it is ≥25 it is recommended to count in groups of five[40].

Horn fly density is usually highest in the scapular, interscapular and costal regions[40] (Figure 2). Also, the back, flanks, legs and both sides of the head can also be considered[35]. Quantification of fly infestation can be done in confined, semi-confined or free-ranging animals[16,34,36].

Figure 2: *Haematobia irritans* infestation on the upper neck and scapular areas of a dark-skinned bull.

Source: Ma. Lorena Torres-Rodríguez

The use of photographs and videos (e.g. VID)[35,36] and videos alone[41] provide the opportunity to very accurately count flies in the recorded images since counting using images is less prone to estimation errors and does not require intensive labor[36]. However, the DV method is faster and more efficient, and sufficiently accurate to identify changes in *H. irritans* population density[39].

Several studies worldwide have estimated that the ET of *H. irritans* in beef cattle is ≥200 flies per animal[16,42]. Exceeding this ET can lead to losses; for instance, it has been estimated that with infestations higher than 200 flies/animal losses of 520 ml milk per day and 28 g live weight per animal per day are produced[43]. Calves and dairy cows cannot tolerate large numbers of flies without experiencing harm. The ET in dairy cows is considered to be no more than 50 flies/animal. The ET can vary between breeds and sexes. For instance, in Holstein breed, the ET is 80-100 flies per animal[44], while beef cattle can tolerate more than 200 flies per animal, although bulls can tolerate even more[45]. In Mexico, the ET is generally estimated by DV. The highest reported fly counts in the country are 120 flies/animal in central Mexico and 300 flies/animal in the southeast, both of which occurred during periods of maximum rainfall[2].

Chemical control and application methods

Chemical control

The most widely used method to control infestations by *H. irritans* in cattle is the use of insecticides. These insecticides are divided in nine main families:

Organophosphates (OPs). Phosphoric acid derivatives interfere with nerve function at the synaptic level by inactivating acetylcholinesterase (AChE), which reacts with serine residues located at the site of AChE catalysis. Because acetylcholine is not hydrolyzed, OPs accumulate excessively, generating an increase in stimulation with an eventual insect paralysis[46]. This mechanism makes OPs highly toxic to animals and humans. OPs are effective against animal ectoparasites such as flies, fleas, lice, mites and ticks, and were the first insecticides used to control *H. irritans*. The most commonly applied OPs compounds are diazinon and ethion, both generally used to control pyrethroid- resistant *H. irritans* populations[33]. Ear tags containing 21.4 % diazinon produced an 87 % reduction of *H. irritans* in grazing cattle in Tuxpan, Veracruz, for up to 90 d[47].

Pyrethroids (Ps). Ps are derived from pyrethrins and are natural insecticides found in *Chrysanthemum cinerariaefolium* flowers. They are classified into TI and TII pyrethrins. TI pyrethrins lack the α-cyano group located at the phenyl-benzyl alcohol position of TII pyrethrins. Natural pyrethrins are sensitive to sunlight, while synthetic Ps are not[48]. Target sites for Ps are the sodium and chloride channels at the point where they inhibit transmission of nerve impulses in insects, causing changes in membrane permeability[33]. The TI Ps change the arrangement of sodium channels in neuronal membranes in response to stimuli, while TIIs affect chloride channels, including those dependent on gamma amino butyric acid (GABA), resulting in membrane depolarization and suppression of the action potential[48]. Insects have a large number of sodium channels sensitive to their structures and body temperature, making Ps highly toxic, in comparison to mammals, where toxicity is minimal[48].

Phenylpyrazolones. These are phenyl pyrazole-type chemical components, and the principal one used in fly control is fipronil. These pesticides act on GABA receptors, blocking chloride channels. They also block two types of chloride channel glutamate activators found only in invertebrates, causing arthropod paralysis and eventually death. A 1 % fipronil-based backsplash formulation shown >80 % efficacy against *H. irritans* up to 21 d after treatment[49].

Macrocyclic lactones (MLs). The MLs are divided into three families: a) the avermectins, which are fermentation products of *Streptomyces avermitilis*, for example, ivermectin, doramectin and eprinomectin; b) the milbemycins, derived from fermentation of *S. cyanogriseus*, for example, moxidectin; and c) spinokines, derived from *Saccharopolyspora*, for example spinosad[50]. The MLs irreversibly interact with GABA and chloride channel glutamate receptors, increasing membrane conductivity and causing paralysis in insects and mites[51]. Because they are effective against endo- and ectoparasites, they are known as endectocides. The chemical composition of avermectins and milbemycins is not altered during passage through the digestive tract and they are excreted intact, meaning that they continue affecting larval development in the manure of treated animals. However, they are also eliminated in milk, which is their main disadvantage[33]. In grazing cattle in Tuxpan, Veracruz, injectable ivermectin has shown >90 % efficacy on fly reduction for up to 90 d after treatment[47].

Growth regulators (GR). In insects, GRs accelerate or inhibit essential physiological processes required for normal development of adult insects and/or progeny. They are not necessarily toxic, but cause abnormalities that compromise insect survival[2]. For example, insect-specific juvenile hormones (JH), which are ecdysone analogues, normally decline in each evolutionary phase, allowing development of adults. Constant JH levels block maturation in insects[52]. The GRs metropene and cyromazine are non-toxic to mammals and are applied via bolus or food supplement in cattle.

Growth inhibitors (GI). GIs block polymerization of N-acetylglucosamine, thus preventing synthesis of chitin, an essential insect exoskeleton component, and as consequence emergence of *H. irritans* does not occur[53]. This group includes benzoyl-phenyl ureas such as diflubenzuron, lufenuron, and triflumuron, of which diflubenzuron is the most widely used against *H. irritans*. These products act against eggs and larvae, not against adult phases. They are usually administered orally, as bolus or as a supplement in mineral salts. Also, spray and powder formulations exist. In the US and Brazil, diflubenzuron produced from 90 to 99 % reduction of *H. irritans* 20 to 33 days after treatment[53]. In Mexico, oral diflubenzuron is used (1 g/animal/day) with good results for *H. irritans* control.

Pyrrole derivatives. Halogenated pyrroles are aromatic organic compounds produced by *Streptomyces*. They are also known as proinsecticides, because, once inside the insect, they are activated by oxygenases such as cytochrome p450 to form more toxic metabolites. Pyrrole targets the mitochondria, affecting oxidative phosphorylation, breaking the proton gradient and preventing production of ATP[54]. A member of this group is chlorfenapyr, the first insecticide used at 30 % in ear tags to control *H. irritans*, and widely used as an alternative treatment for pyrethroid-resistant *H. irritans*[33].

Repellents. Some plant extracts and essential oils, mainly nitrogenous compounds, alkaloids, phenolics, protein inhibitors and essential oils[55], exhibit insect repellent activity. They represent a replacement for use of conventional insecticides in organic production units, or an alternative to conventional pest control methods that can help mitigate insecticide resistance. One limitation of extracts or essential oils is their short repellence effect. For example, the essential oils of lemongrass (*Cymbopogon citratus*), geranium (*Geranium odoratissimum*), and peppermint (*Menta piperita*) at 5 % concentration in sunflower oil, exhibited repellence during 8 to 24 h[56] versus *H. irritans.*

Attractants. These are volatile substances detectable by insects over large distances and emitting alarm or reproductive signals. In the case of *H. irritans*, they are pheromones or chemical messengers found in the cuticular wax of females. This cuticular wax is composed of 21- to 29-carbon chains which function as copulation stimulants for males[2]. Synthetic pheromones have been applied in traps treated with insecticides to attract insects, but in this way, they have functioned as physical rather than chemical control method[57].

Application methods

Several methods of application of insecticides to control *H. irritans* exist. The method of choice depends on factors such as farm type, production system (intensive, extensive, mixed), beef and dairy cattle, or both), excreta management, infrastructure, facilities, and the technical personnel in charge of insecticide application[2]. The most common methods of application of insecticides are described below[3,58,59].

Insecticide-impregnated ear tags: These are plastic ear tags containing one or more insecticides in the tag matrix. As the tag moves small amounts of insecticide are released and distributed through the animal's hair. Ear tags are currently available containing Ps, OPs, MLs, and Ps/OPs mixtures. All adult animals in a herd should be tagged, and tags should be removed if no efficacy is observed.

Powders: Powdered insecticide is placed in sacks or bags from which small amounts are released through filters when an animal is in contact with the bag. Using this method requires that bags are suspended near water intakes and arranged in a way that ensures that the dust falls onto the animals. Powdered insecticide is also used to treat manure.

Dorsal pour-on: Dorsal pour-on insecticides are applied along an animal's back line, at a weight-dependent dose. This is one of the most widely used methods for cattle in Mexico.

Sprays: Spray treatments effectively control flies, but the insecticide must be applied on the entire animal. This method increases animal handling and stress in animals, which is a disadvantage. However, it is effective when small numbers of animals are treated. Spraying is a common method used in Mexico.

Oral larvicides: These are directly applied in food, mineral blocks, or as food supplements. Oral insecticides include MLs, GRs and GIs. They pass through the gastrointestinal tract and are excreted in the feces where they prevent larval development. One challenge with this technique is ensuring that sufficient active agent is applied, because underdosing may allow fly infestation levels above the ET. A solution to this challenge is to use slow-release boluses, which remain in the reticulum, and continuously release the product.

Injection or systemic: Although the vast majority of insecticides are applied topically, intramuscular injection is effective for applying of MLs such as ivermectin. This is a very common method used to control ticks, flies and gastrointestinal nematodes in beef cattle.
VetGun®: This novel insecticide administration method involves firing an insecticide-loaded gelatin capsule (VetCap®) from a special gun. The capsule is very fragile and breaks upon impact with the skin, releasing the insecticide which begins disseminating through the animal's hair and skin. Capsules can be shot onto an animal from 5 to 10 m away, although it does not ensure the insecticide adequately covers both sides of the animal. This technology is not yet commercially available in Mexico, but may become more available in the near future.

Bioinsecticides. These are extracts or essential oils from plants that have efficacy on the control of *H. irritans*. For example, the development of *H. irritans* in feces was inhibited by an extract from neem (*Azadirachta indica*) containing azadirachtin administered orally to cattle at doses of ≥0.03 mg per kg body weight per day in a food supplement of neem seeds at ≥ 10 mg of seeds per kg[60]. Other botanical compounds with good efficacy against *H. irritans* are p-anisaldeide, extracted from plants such as *Pimpinella anisum* and *Cuminum cyminum*[61], and essential oils of *Carapa guianensis*[62], *Eucalyptus polybractea*[63], and *Pelargonium* spp.[56].

In a study conducted in dairy cows naturally infested with *H. irritans* in Mexico, a reduction of infestation from 9.5 to 68.0 % was found after spraying 20% *Larrea tridentata* leaf extract[64]. Further research is needed in Mexico to identify bioactive molecules in extracts from native plants from different regions in the country, and to develop vehicle formulation and application methods in cattle.

Insecticide resistance in *H. irritans*

Insecticide resistance is a genetic-evolutionary response of insect populations exposed to continuous stress due to frequent insecticide exposure. In the field, resistance is suspected when a previously effective product no longer demonstrates the same effect; this applies as long as the application and dose have been optimal[42]. Because the *H. irritans* life cycle lasts few days, control treatments are carried out at short intervals, leading to a progressive increase in the frequency of resistant individuals and eventual loss of insecticide biological effectiveness[20].

Several resistance mechanisms in *H. irritans* are known. They include changes in insect behavior to avoid insecticide exposure, detoxification by overexpression of the cytochrome p450 enzyme, and insensitivity at the site of action due to mutations in the sodium channel[42]. Resistance to Ps in *H. irritans* is associated with resistance to knockdown due to mutations in the sodium channel (known as kdr or super kdr) which prevent or reduce interaction with the sodium channel[65]. Resistance to OPs arises from point mutations that produce changes in acetylcholine's structure, conformation and site of action. These changes have been found in the active site of AChE in OPs-resistant mosquitoes, and are known to result in decreased AChE sensitivity[33].

Insecticide resistance is most commonly diagnosed using a bioassay in which recently captured flies are exposed to filter papers impregnated with insecticide at lethal concentrations (LC) of 0, 50 and 99 %, using acetone as a diluent. Three replicates are done and after one hour of exposure, the percentage of mortality is recorded for each concentration in comparison with the control (100 % acetone)[66]. Resistance to Ps can be identified molecularly. A fragment of the gene that codes for the sodium channel of a single individual is amplified and the resulting sequences analyzed, identifying whether the mutations are kdr or super-kdr type. For OPs, PCR is used to identify a point mutation where a glycine is substituted for an alanine at position 262 of the AChE amino acid sequence. With this method, fly resistance can be detected in the field[67].

H. irritans resistant to insecticides has been documented in the US since the 1960s. Resistance to Ps in *H. irritans* populations controlled by Ps ear tags was first reported in the 1980s in Florida, US. The first study of resistance in *H. irritans* in Mexico was done on the Gulf of Mexico, finding high resistance to fenvalerate and less resistance to OPs[68]. A study to test the susceptibility of *H. irritans* to cypermethrin and diazinon in the state of Tamaulipas, Mexico, detected the presence of both kdr and super-kdr genes. The super-kdr gene was only identified at one ranch, but kdr frequency ranged from 43 to 78 % in the remaining studied places[66]. Another study done in Tamaulipas used filter paper tests to

confirm that Ps resistance in *H. irritans* was distributed across the state, but simultaneous resistance to Ps and diazinon was only found in populations in the south[69]. Similar studies showed high resistance to Ps and low resistance to diazinon in northern Veracruz central Nuevo León[70]. In Guerrero, resistance to both OPs and Ps was found in 100 % of 30 sampled farms[71]. Currently, the geographical distribution of *H. irritans* resistant to the main insecticide families is unknown in Mexico, highlighting the need for a national-level resistance survey.

Alternative control methods

Physical control. Physical control of *H. irritans* involves trapping adult flies as they search for new cattle hosts. Some traps are cylinders or inverted cups in shaped, and are covered with a sticky material, others are like black balls and emit violet light, and others are impregnated with attractants such as pherohormones[72]. Another kind of physical control is the walk-through trap, which consists in a dark tunnel. As the animals walk through the dark tunnel, flies separate from it seeking lighted areas on the roof, where they are trapped and die within 2 to 12 h. Some walk-though traps are equipped with an electric suction system to vacuum the flies. However, this requires electric installation and this increases the operation costs[34]. The use of traps is very limited in Mexico, therefore this is an area of opportunity for development and evaluation. Physical control of *H. irritans* reduces the use of insecticide, and selection of insecticide-resistant horn-fly populations.

Biologic control. The use of natural enemies of *H. irritans* is a widely explored way to control its populations[73-76]. Natural enemies of *H. irritans* include Pteromalid parasitoids, such as the genera *Muscidifurax* spp. and *Spalangia* spp., that parasitize fly pupae; entomopathogenic bacteria such as *Bacillus thuringiensis*; entomopathogenic nematodes such as *Steinernema* spp. and *Heterorhabditis* spp.; and entomopathogenic fungi such as *Beauveria* spp., *Metarhizium* spp. and *Isaria* spp. Dung beetles of the Scarabaeidae family also play an important role in biological control of *H. irritans* by degrading cattle feces and incorporating them into the soil, thus preventing development of the non-parasitic phase of *H. irritans*[50]. Biological control strategies pose minimal risk to non-target invertebrates and vertebrates (including birds, and mammals), while reducing insecticides and development of horn-fly resistance[77,78].

Parasitoids attack any fly species and are available in the Mexican and international markets for use in livestock production systems. They are sold in cloth bags or plastic containers containing housefly pupae parasitized by one or two genera of wasps (*Muscidifurax* and/or *Spalangia*). These are placed in paddocks and pens 48 hours before emergence of the adult

parasitoids, which easily establish themselves in environments with moderate chemical product use. Various parasitoid species have been reported in Mexico, the most frequent being *Spalangia endius, S. nigroaenea,* and *Muscidifurax raptor*[73].

When applied directly to manure, entomopathogenic bacteria such as *B. thuringiensis* is useful in controlling larval-stage *H. irritans*. However, limited data is available on its use in the field. Entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis* spp.) have been presented as an alternative method of biological control, but further research is required to evaluate their use in the field[74,76].

In Mexico, various isolates of *B. bassiana* (*Bb*), *M. anisopliae* (*Ma*), and *Isaria fumosorosea* have been evaluated *in vitro* versus *H. irritans*[75]. A study performed under controlled conditions in the dry tropics, evaluated different formulations on cattle, found out that five *M. anisopliae* strains controlled 94 to 100 % of infestation after 12 to 13 days' post-treatment, while three *I. fumosorosea* strains decreased generation of immature phases from 90 to 98 % up to 13 days' post-treatment[79].

An aqueous formulation of the Mexican strain Ma134 of *M. anisopliae* evaluated in dairy cattle naturally infested with *H. irritans* in a semi-arid climate controlled 68 % of the infestation after four weeks' treatment[80]. Strain Ma135 was evaluated against natural infestations of *Stomoxys calcitrans* and *H. irritans* in dairy cattle in a combined grazing/corral system, lowering the *S. calcitrans* infestation by 69% and the *H. irritans* infestation by 58 % at six weeks' treatment[81]. The main disadvantage of entomopathogenic fungi treatments is that ultraviolet rays deactivate conidia. Therefore, application of entomopathogenic fungi must be done before sunrise to maintain its efficacy.

Dung beetles form the Scarabaeidae family degrade organic matter in feces, competing with *H. irritans* for space and organic matter. During their mating process, these beetles bury feces in the soil, preventing horn flies from development. Under laboratory conditions, the *Aphodius lividus* beetle is capable of reducing *H. irritans* emergence by 98 to 100 %[82]. A study performed in North America found that a density 40> of *Digitonthophagus gazelle* adult beetles in cattle feces reduced the emergence of *H. irritans* from 38 to 56 %[83]. However, it is known that dung beetle populations are negatively affected by MLs, such as ivermectin and doramectin[59,84]. For example, use of 10 % moxidectin in cattle reduces reproductive capacity in the dung beetle *Onthophagus landolti*[85]. The challenge is to use selective treatments that generate lower ML excretion levels, and consequently lower the impact on dung beetle populations.

Immunological control. The need for horn-fly control methods friendy with the environment and public health has encouraged research on the immune response of cattle to *H. irritans* antigens for anti-horn fly vaccines, analogous to the approach used with ticks. It has been demonstrated that 200 flies per animal produce a weak antibody response to antigens from fly saliva, increasing when the flies are removed from the animals. This suggests a modulation effect of the antigens in the *H. irritans* salivary glands[86]. Another study identified a correlation between reductions in egg counts and levels of antibodies against *H. irritans* fed with blood from bovines immunized with antigens from *H. irritans* intestine; however, the fly mortality was not significant[87].

Vaccination of cattle with recombinant proteins such as thrombostasin, a coagulation-inhibiting protein identified in the salivary glands of *H. irritans*[88], and hematobin, an immunomodulatory protein from saliva, produced a decrease in blood consumption by flies, and decreased development of eggs, and adult flies. Experimental vaccination with recombinant hematobin increased the anti-hematobin IgG response in cattle and reduced fly numbers in 30 % compared to controls[4]. So far, very few recombinant proteins have been evaluated and a recombinant vaccine against *H. irritans* does not exist yet.

Functional genomics and proteomics studies offer an opportunity to discover new candidate vaccine antigens that can then be expressed and produced in recombinant proteins to be used alone or in combination as part of vaccination and challenge trials against *H. irritans* infestations in cattle. In Mexico, candidates for *H. irritans* vaccine development were identified via gene silencing using RNA interference (RNAi) in a cDNA library constructed from abdominal tissues of partially fed *H. irritans*. The RNAi of the protease inhibitor functional group produced high mortality and vitellogenin, ferritin, and ATPase, as well as components of the proteasome, immune response and 5'-NUC produced reduction of oviposition of. However, these candidates have not been evaluated in immunization against *H. irritans* and infestation trials[89].

Little research on identification of candidates for development of vaccines against *H. irritans* has been performed and so far, the results are preliminary. Therefore, the immunological control of horn fly is not an alternative in the short-term. The recent sequencing, assembly and annotation of the *H. irritans* genome[90] will be useful on identification of new candidate antigens for vaccine development.

Cultural, tactical, strategic, and selective control

Cultural control is the implementation of good management practices such as the removal and proper disposal of fresh excreta from pens and stables, which interrupts the horn fly life cycle and prevents development of new populations[42].

Tactical control is an immediate action triggered by harmful infestation levels. Effective tactical control requires monitoring of the fly population every 8 to 10 d with immediate treatment when infestation levels exceed the ET[2].

Selective control is to apply treatment of only those animals with the highest fly infestation levels in a herd. Several trials applying different insecticides to 25 % and 50 % of the herd reduced infestation levels of *H. irritans* in the herd with a low cost; however, more frequent treatments were required due to fly infestation persistence[91].

Strategic control is based on knowledge of the epidemiology and biology of *H. irritans* in a given region. In this case, limitation of treatments during highest infestation and economic damage are applied to prevent peaks in fly populations. This approach can be implemented once a pre-established maximum fly infestation level is exceeded based on weekly evaluations[2].

Integrated control

Integrated pest control (IPC) considers the association between the environment and population dynamics of parasite species, using a combination of compatible techniques and sustainable methods to maintain parasite populations below the ET. Application of IPC is generally associated with a drastic decrease on frequency of treatments and as consequence the genetic selection pressure and resistant parasites decrease[1]. Although different strategies to control *H. irritans* have been explored worldwide, no research has been done on integrating strategies, in contrast to other parasites such as ticks[92]. In Mexico as in other countries, the main challenge on *H. irritans* control is to design and establish effective IPC that include chemical and non-chemical strategies.

Conclusions

Based on the information presented and discussed on the situation and prospects for the study of *H. irritans* in cattle in Mexico, it is concluded that:

Horn fly *H. irritans* is an obligate ectoparasite of cattle, that is distributed across Mexico, during the year, with peaks in summer or in rainy season. This parasite is responsible for significant economic losses in cattle systems, highlighting the need to study its population dynamics in different regions of the country, to establish effective control strategies and prevent population peaks.

Chemical methods are the most common approach to control *H. irritans* infestations. Insecticides used to control these flies include OPs, Ps, ML, GR, GI, and pyrroles, as well as repellent and attractant products. Insecticides are applied using various methods and application ways. The frequent use of insecticides selects genetic resistant populations of *H. irritans.* In Mexico, populations of *H. irritans* resistant to OPs and Ps have been reported in the states of Tamaulipas, Veracruz, Nuevo León, Guerrero and in southeastern Mexico.

Biological control is a promising alternative from which entomopathogens fungi is the most useful method. The species *B. bassiana, M. anisopliae* and *I. fumosorosea* have been shown efficacy against *H. irritans* in Mexico. Another approach of biocontrol is the use of dung beetles that degrade organic matter in feces and compete for resources blocking development of immature *H. irritans* stages. The frequent application of MLs for control of endo and ectoparasites negatively affects dung beetle development, therefore, rational use of ML in cattle systems to preserve natural regulators of *H. irritans* populations is needed.

Research is required on several areas to find other ways to control *H. irritans*, emphasizing on the identification and development of new bio-insecticides, and the use of integrated control strategies.

Studies are required to identify and develop new bioinsecticides for the control of *H. irritans* in cattle.

The use of different integrated control strategies for *H. irritans* has been little explored worldwide and in Mexico.

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