

## EDITOR'S CHOICE

# Traumatic myiasis by *Wohlfahrtia magnifica* in sheep flocks from southeastern Spain: prevalence and risk factors

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**Abstract.** *Wohlfahrtia magnifica* (Diptera: Sarcophagidae) is an obligate myiasis-causing fly of livestock that is widely distributed throughout the Mediterranean basin. A total of 73 683 sheep from 122 flocks in Albacete Province (southeastern [SE] Spain) were examined to detect active traumatic myiasis. The influence of several individual and flock factors on the prevalence was also assessed. The overall flock prevalence of traumatic myiasis was 95.9%, with an individual prevalence of 7.1%. *Wohlfahrtia magnifica* was identified in all of the positive animals except one that was infested by *Lucilia sericata* (Diptera: Calliphoridae). Adults of both genders showed a higher risk of infestation than juveniles, whereas males of all ages displayed an increased risk of infestation than females of all ages; the existence of a lambing building on the farm, poor husbandry facilities and the location area were also identified as risk factors. Prevalence was highest in the summer, and the fly season lasted from March to November. Genitalia were the main location of infestation in males (94.4%) and females (76.3%). Our results reveal that traumatic myiasis by *W. magnifica* is widespread in southeastern Spain, hence preventive measures minimizing the attractiveness of sheep to gravid flies are necessary to avoid health and welfare problems and significant production losses in sheep flocks.

**Key words.** *Wohlfahrtia magnifica*, myiasis, risk factors, sarcophagidae, sheep, southeastern Spain.

## Introduction

*Wohlfahrtia magnifica* (Schiner, 1862), one of the species referred to as flesh flies, is an obligate agent of traumatic myiasis in livestock (Hall & Farkas, 2000). Larval stages primarily feed on the tissue of living mammals, although they have been occasionally found in birds, and human cases have also been reported (Karaman *et al.*, 2009; Robbins & Khachemoune, 2010). *Wohlfahrtia magnifica* is widely distributed throughout the Mediterranean basin, Eastern and Central Europe, Northern Africa and Central and Northern Asia

(Hall & Farkas, 2000; Tligui & Zerhouni, 2010; Giangaspero *et al.*, 2011, 2014). In these regions, wohlfahrtiosis is related to the loss of animal condition, reproduction disorders, lameness and blindness that could be fatal if untreated; hence, this myiasis leads to important welfare and health problems and significant economic losses (Hall & Wall, 1995; Farkas *et al.*, 1997).

In contrast to other traumatic myiases, prior wounding is not a prerequisite for infestations with *W. magnifica* (Farkas *et al.*, 1997). Females are attracted to both wounds and natural body orifices (ears, eyes, nose and genitalia) of their hosts, where they deposit their first instar larvae (Goddard, 1996). Larvae

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feed for 5–7 days, causing significant tissue destruction, and after two moults (L1–L3), they leave the wound, fall to the ground and pupariate (Goddard, 1996). Most topically applied curative insecticides are effective in killing *W. magnifica* larvae, but they do not provide long-term protection from infestation or re-infestation (Sotiraki *et al.*, 2005).

Sheep are considered major hosts of *W. magnifica* (Hadani *et al.*, 1971; Hall, 1997), with individual prevalences ranging from 0.7 to 95% in Europe (Hall & Farkas, 2000; Sotiraki *et al.*, 2012). Spain has the largest sheep population in the European Union, and Castilla-La Mancha (SE Spain) is a region where sheep farming, considered a strategic economic sector, has a long tradition. Although the economic impact of traumatic myiasis is significant in Spain (Ruiz-Martínez *et al.*, 1987; Ruíz-Martínez *et al.*, 1992, 1993; Ruíz-Martínez & Leclercq, 1994), information regarding its prevalence and predisposing factors in many areas of the country is still limited. The aim of this study was to determine the prevalence of traumatic myiasis in sheep from SE Spain and to assess the possible influence of some internal (age and sex) and external (geographical location, farm facilities and flock management) variables on the percentage of infested sheep or other domestic animals on the farms. In addition, the seasonality and anatomical distribution of larval infested cutaneous lesions were evaluated.

## Materials and methods

### Study area and flocks

A cross-sectional study was conducted in Albacete, a province with a total area of 14 858 km<sup>2</sup> located in Castilla-La Mancha (SE Spain). This province has a Mediterranean climate with a substantial Continental influence characterized by cold winters and hot summers. The annual average temperature is 13.4 °C, with a thermal range of 19.3 °C and annual average precipitation of 343 mm. According to temperature and precipitation records, three areas can be defined in Albacete province (Fig. 1): (a) the northern plateau (NP) area with the driest and coldest conditions, and the southern mountainous area divided into (b) the western Continental-mountainous (CM) area, characterized by high precipitations during winter and spring and (c) the eastern Mediterranean-mountainous (MM) area, which registers the highest precipitation during autumn.

A total of 646 893 sheep located in 1617 flocks were registered in Albacete province in 2007 (MAPA, 2010); most of them are devoted to meat production and managed under an extensive husbandry system.

### Sampling

A total of 73 683 sheep, including adults (ewes and rams) and juveniles (replacement lambs), from 122 flocks were sampled in 2011. Replacement lambs were animals older than 3 months of both genders bred on the farm to replace non-productive animals. The flocks were classified according to the management system as intensive (breeding, management and feeding of the animals occur inside the buildings), extensive (breeding,

management and feeding of the animals occurs at grazing areas) and semi-extensive (alternation between extensive and intensive management practices).

Most sampled animals were adults in production (88.1%) and females (85.8%) of the autochthonous Manchega breed (75.2%) (Table 1). Other domestic species on the farms, such as dogs, cats and cattle, were also examined for the presence of traumatic myiasis.

All the farmers completed a survey every month compiling information about the presence of infested animals on the farm. The farms were analysed between April and June, when a careful visual examination of the animals was performed, and all the larvae were removed with forceps and stored in 10% formalin solution. The location of the larvae was registered, considering 15 different anatomical areas: head/neck, mouth/nose, eyes, ears, rump, ribs, chest, abdomen, thigh/hind legs, shoulder/forelegs, feet, tail, preputial area, vulvar and/or perianal region and udder (Fig. 2). After removing the larvae, wounds were cleaned and treated with a pyrethroid spray in aqueous solution.

### Larvae identification

Larvae identification was performed on third instars based on the pattern of spinulation and morphological examination of the cephalopharyngeal skeleton and anterior and posterior spiracles. For this purpose, larvae were boiled in 10% potassium hydroxide for 10 min and subsequently examined under a stereomicroscope using morphological keys (Zumpt, 1965; Ruiz-Martínez *et al.*, 1990).

### Statistical analysis

To perform a risk factor analysis, a questionnaire was conducted for each flock to gather information on different inherent characteristics of sheep (age and sex); data on the production system, flock size, management, facilities and control strategies were also included. All factors considered and the distribution of both flocks and animals in each category are summarized in Table 1.

Mixed-effects logistic regression was used to identify the factors significantly favouring individual infestation; flock was considered as a random factor to control the effect of different flock sizes over the final model. Both individual and farm factors were included in the analysis. This analysis was performed with the `glmer()` function from the `LME4` package (Bates *et al.*, 2015) in the `R` statistical package (`R` v.4.0.3; `R` Core Team, 2020). Finally, a logistic regression was used to estimate the association of wohlfahrtiosis in other animal species on the farms with the different variables considered.

In logistic regression, factors were eliminated from the initial model using a backward–forward conditional method based on Akaike Information Criterion value (AIC) until the best model was built. Odds ratios were computed by raising *e* for the estimation of the logistic coefficient over the reference category. These statistical analyses were performed with the `glm()` function in the `R` statistical package (`R` v.4.0.3; `R` Core Team, 2020).

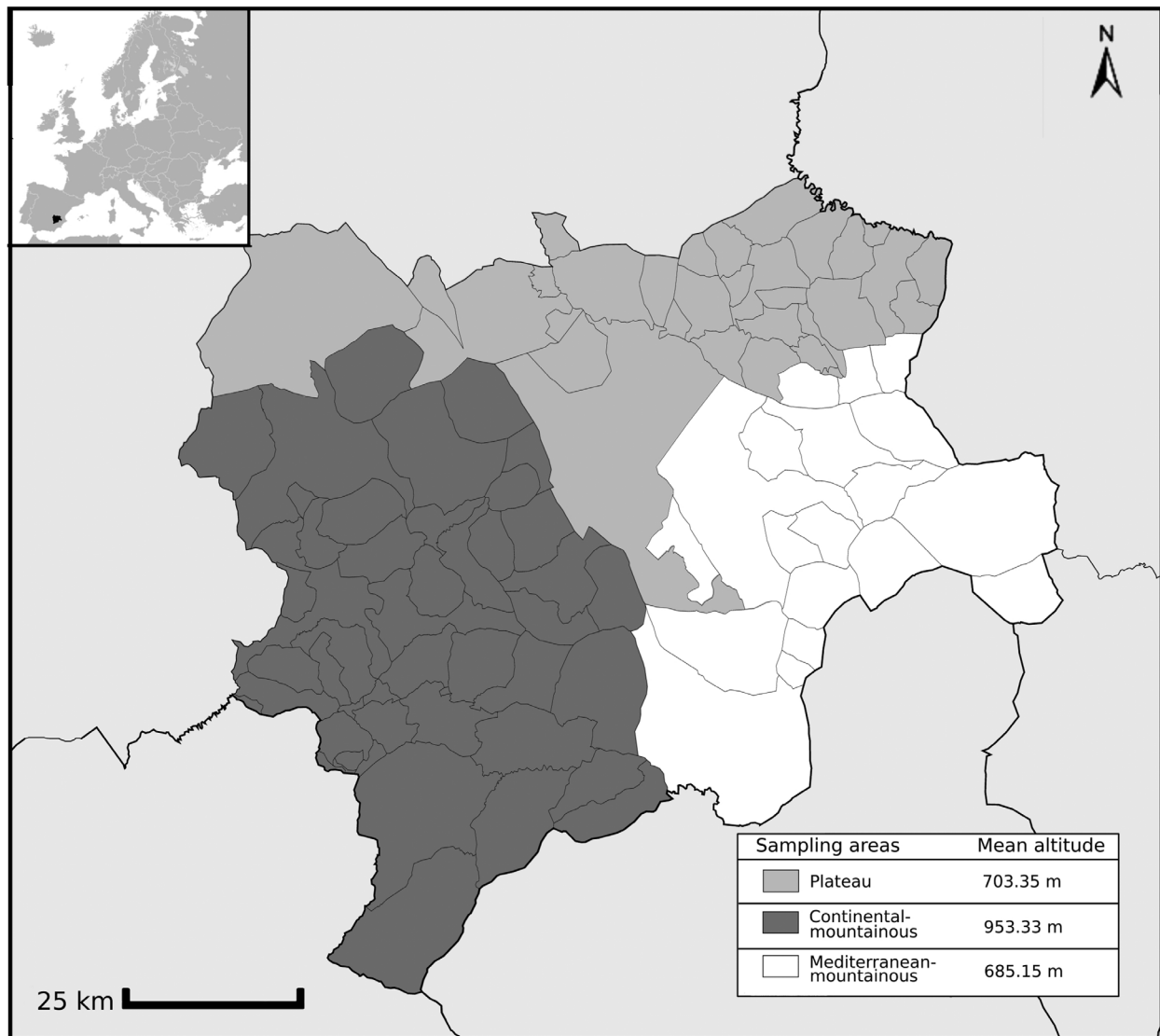


Fig. 1. Map of Albacete province showing the location of the three geoclimatic areas considered in the study.

## Results

### *Flock prevalence and risk factor assessment*

The overall flock prevalence of cutaneous myiasis was 95.9% (117/122), with a mean intra-flock prevalence of 7.9% (range 0–32.1%). Table 1 summarizes the prevalence at flock level according to the different variables considered in the study. Regarding the management, all flocks under an extensive grazing system ( $n = 90$ ) were positive for traumatic myiasis, whereas none of those maintained in an intensive management system ( $n = 3$ ) was positive. The percentage of positive meat flocks (96.6%) was higher than that of dairy ones (93.9%), and flock prevalence in CM (97.9%) and MM (97.1%) areas was very similar and higher than that in NP (92.5%) area.

Infestations by *W. magnifica* in other domestic animals were detected in 76 farms (62.3%) and included goats, cows, dogs,

horses and donkeys; one case of a human infestation was also reported. Logistic regression showed that the presence of traumatic myiasis in other animal species was related to the number of infested sheep on the farm, antiparasitic bath use and geoclimatic area, with lower percentages in the MM area (Table 2).

### *Individual prevalence and risk factor analysis*

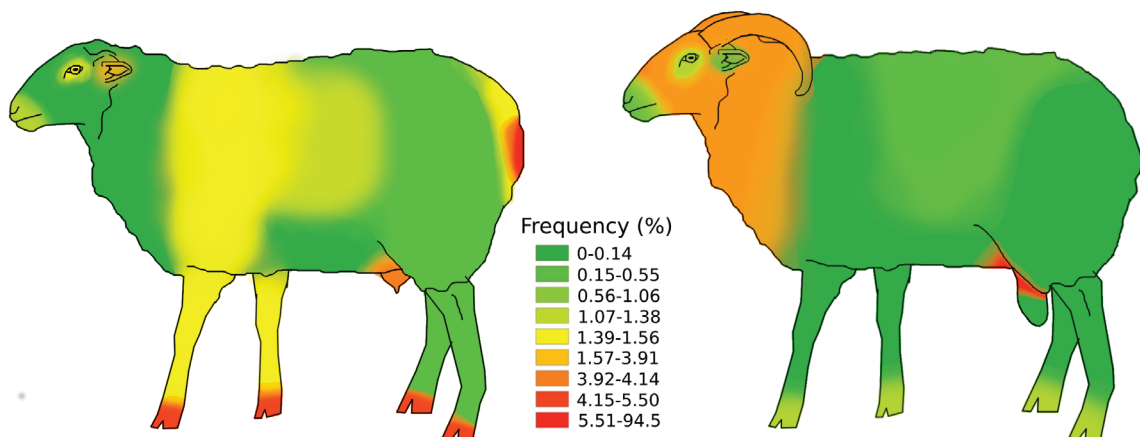
Overall, 5284 out of 73 683 animals (7.2%) presented cutaneous myiasis; all animals but one were infested by *W. magnifica* (99.98%), representing a total individual prevalence of 7.1%. A single sheep was infested by *Lucilia sericata* (0.02%).

The risk analysis (Table 3) identified the age, sex, production system, the existence of a lambing building, the quality of farm facilities and the geoclimatic area as significant risk

**Table 1.** Distribution of the samples according to the variables included in the study and individual and flock prevalence of cutaneous myiasis by *Wohlfahrtia magnifica*.

Factor	Category	Flock		Sheep	
		N	Prevalence (%)	N	Prevalence (%)
Age	Adult	—	—	64 947	8
	Lamb	—	—	8736	0.2
Sex	Female	—	—	71 597	5.7
	Male	—	—	2086	53.7
Production system	Dairy	33	93.9	34 170	7.3
	Meat	89	96.6	39 513	6.9
Flock size	≤250	43	93.0	5888	8.0
	251–499	35	100.0	14 926	8.1
	≥500	44	95.5	52 869	6.6
Geoclimatic area	NP	40	92.5	31 350	7.1
	CM	48	97.9	21 778	7.8
	MM	34	97.1	20 495	6
Management system	Intensive	3	0.0	654	0
	Semi-extensive	29	93.1	30 764	7.1
	Extensive	90	100.0	42 265	7.2
Other species infested	Yes	77	93.5	51 365	7.4
	No	45	100.0	22 318	6.2
Lambing building	Yes	109	100.0	62 820	7.2
	No	13	61.5	10 863	6.4
Docking of tails	Yes	112	96.4	65 020	7.1
	No	10	90.0	8663	7.1
Quality of facilities	Poor	22	95.5	6051	9.9
	Acceptable	68	95.6	43 167	7.1
	Good	32	96.9	24 465	6.3
Animals/worker	≤250	44	93.2	6456	8.7
	251–449	36	97.2	26 774	6.7
	≥500	42	97.6	40 453	7.1
Antiparasitic bath	Yes	94	96.8	51 843	6.6
	No	28	92.6	21 840	8
Systemic treatment	Yes	78	97.4	53 382	6.8
	No	44	93.2	20 301	7.7
Topical treatment	Yes	74	97.3	47 836	7
	No	48	93.7	25 847	7.1
Waste container	On-farm	42	95.2	28 672	6.4
	Off-farm	80	96.2	45 011	7.5

CM, Continental-mountainous; MM, Mediterranean-mountainous; NP, northern plateau.

**Fig. 2.** Frequency of *Wohlfahrtia magnifica* infestation considering the anatomical distribution in females (left) and males (right).

**Table 2.** Logistic regression model to estimate the association of cutaneous myiasis in other domestic species and the flock prevalence by *Wohlfahrtia magnifica*.

Factor	Category	Estimate	Z value	P value	OR	CI 97.5%
No. of positive sheep	—	0.043	0.01392	0.002	1.04	1.017–1.074
Antiparasitic bath	No	—	—	—	—	—
	Yes	1.357	2.454	0.014	3.88	1.355–12.069
Geoclimatic area	MM	—	—	—	—	—
	NP	1.836	0.589	0.002	6.27	2.071–21.207
	CM	1.337	0.506	0.008	3.81	1.444–10.632

CM, Continental-mountainous; MM, Mediterranean-mountainous; NP, northern plateau.

**Table 3.** Risk factors associated with individual prevalence of *Wohlfahrtia magnifica* based on the final mixed multivariable logistic regression model.

Factor	Category	Estimate	Z value	P value	OR	CI 95%
Age	Lamb	—	—	—	—	—
	Adult	4.485	18.605	<0.001	88.70	55.298–142.31
Sex	Female	—	—	—	—	—
	Male	3.546	61.750	<0.001	34.69	31.001–38.829
Geoclimatic area	MM	—	—	—	—	—
	NP	0.289	1.53	0.126	1.33	0.922–1.933
	CM	0.452	2.522	0.012	1.57	1.106–2.235
Production system	Meat	—	—	—	—	—
	Dairy	0.346	2.057	0.040	1.41	1.016–1.965
Lambing building	No	—	—	—	—	—
	Yes	0.954	3.39	<0.001	2.60	1.495–4.514
Quality of facilities	Good	—	—	—	—	—
	Acceptable	0.067	0.397	0.062	1.07	0.766–1.494
	Poor	0.445	2.034	0.042	1.561	1.016–2.398

CM, Continental-mountainous; MM, Mediterranean-mountainous; NP, northern plateau.

factors. Thus, adults were 88 times more likely than replacement lambs to be infested by *W. magnifica*; moreover, rams showed a risk of being infested 34.7-fold higher than ewes. In relation to the production and husbandry system, dairy sheep were 1.41 times more likely to present traumatic myiasis than meat-producing animals; animals from farms with a lambing building had 1.5-fold higher odds of being infested than those from farms without such facilities. Significant differences between good and old and poor husbandry facilities were also found. Finally, the highest prevalence was registered in the NP area (9.85%), followed by the CM (7.78%) and MM (6.01%) areas. Compared to the MM area, the CM area had the largest odds ratio for reporting *W. magnifica* infestation (Table 3).

#### Anatomical distribution

The anatomical distribution of the myiasis showed noticeable differences when considering the sex of the animals (Fig. 2). In females, the most infested region was the vulvar and/or perianal area (76.3%), and only a low percentage presented myiasis in their feet (6.6%), udder (4.2%) and ears (4.1%); less than 0.5% had larvae in thigh or hind legs. No cutaneous myiasis was observed in the abdominal area, chest, head/neck and rump of females. In contrast, the preputial area was the most frequently infested area in rams (94.4%), followed at a substantially lower

frequency by the head/neck (3.3%). In the rest of the anatomical areas, the frequency of infestation was lower than 0.6% (Fig. 2).

#### Seasonal distribution pattern

Based on the monthly farmer surveys, in both mountainous areas (CM and MM), the first traumatic myiasis cases appeared in March, peaked between July and September, and began to decrease in October, disappearing in December (Fig. 3). However, wohlfahrtiosis lasted longer in the plateau area, because the first cases were observed in February and maintained high levels of prevalence until November. No traumatic myiasis cases were detected from December to February in any region.

#### Discussion

The results of the present study reveal that traumatic myiasis is highly prevalent and widely spread in sheep farms from southeastern (SE) Spain; in fact, all extensive flocks, and a high percentage (93.1%) of semi-extensive farms were positive. *W. magnifica* was the overwhelmingly dominant myiasis-causing species, being identified in all but one positive animal, in which *L. sericata* was detected. Our data are consistent with those reported by Farkas *et al.* (1997) in Hungary, where *W. magnifica* ( $n = 4383$ ) was clearly dominant over *L. sericata*, which was only identified in a low number of sheep ( $n = 5$ ).

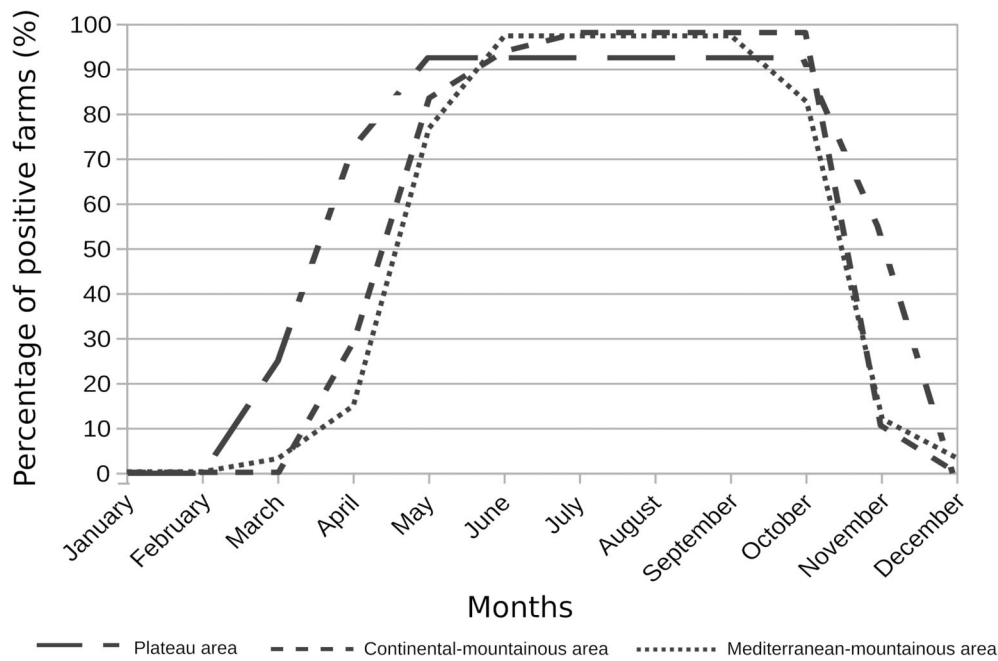


Fig. 3. Monthly distribution of the percentage of positive flocks considering the geoclimatic area.

The high prevalence of traumatic myiasis observed in extensive and semi-extensive farms indicates that grazing animals are more accessible to gravid flies than confined ones, agreeing with previous reports (Ruíz-Martínez *et al.*, 1992; Sotiraki *et al.*, 2003, 2012). However, the absence of traumatic myiasis in intensive farms could reflect the low representation of this type of farm in the study ( $n = 3$ ).

The high percentage of positive flocks (95.9%) contrasts with the low proportion of infested animals per flock (7.2%). In Europe, the prevalence of wohlfahrtiosis in sheep is particularly variable, ranging from 0.7 to 95% (Sotiraki *et al.*, 2012). The highest prevalences (around 36%) have been reported in eastern countries such as Bulgaria, Hungary, Romania, Kazakhstan and Ukraine (Isimbekov & Zhumbekov, 1983; Farkas *et al.*, 1997). Our results, in contrast, are consistent with previous studies performed in western Mediterranean countries (Spain, Portugal, Italy and France), showing individual prevalences of approximately 8% (Ruíz-Martínez *et al.*, 1992; Giangaspero *et al.*, 2011, 2014; Sotiraki *et al.*, 2012). There is strong evidence that in endemic areas, autochthonous breeds are less susceptible to infestation than allochthonous ones, which are more predisposed to infestation (Lehrer & Verstraeten, 1991). In this regard, Farkas *et al.* (1997) showed that the infestation rate in Hungarian flocks was lower in autochthonous (5.8%) than in imported naïve (28.8%) breeds such as Merino, Romney and Corriedale. Livestock owners commented that imported breeds seem to be more individualistic and separated from one another than local sheep, which, by contrast, tend to aggregate during fly attacks (Farkas *et al.*, 1997). Hence, the low individual prevalence found in our study may be the result of the high proportion of autochthonous Manchega breed, which could have developed a defensive behaviour that would prevent infestation; in this regard, Ruíz-Martínez *et al.* (1993) reported that in

autochthonous sheep and goat races about 75% of larviposition attempts by *W. magnifica* failed, indicating that the host defence is highly effective.

Mixed-effect logistic regression identified age and gender as risk factors for wohlfahrtiosis. Adults are significantly more exposed to fly attacks than replacement lambs, possibly because flies are attracted to the sexual secretions of mature animals. Although the female genitalia are predisposed to infestation during mating, due to higher production of vaginal fluids at oestrus, particularly when hormone-impregnated sponges are used (Sotiraki *et al.*, 2003), rams are much more frequently infested than ewes. It has been reported that gravid flies of *W. magnifica* are strongly attracted to male sheep (Ruiz-Martínez *et al.*, 1987; Farkas *et al.*, 1997), an observation that could be related to chemical substances in sexual secretions acting as fly attractants. In addition, the higher prevalence found in males may be associated with their handling, being more complicated, therefore preventing routine inspections or treatments.

Regarding the production and management system, dairy sheep were more frequently infested than meat-producing sheep. In this respect, the incorrect use or design of the milking equipment, as well as the use of suboptimal methods for handling and restraining dairy sheep, may increase the risk of injuries and subsequent infestation. It is worth noting that the effect of this variable on the prevalence of *W. magnifica* was low (OR = 1.41), and the differences were close to being non-significant; therefore, these results could be due to the low number of dairy farms sampled. By contrast, the quality of the facilities is highly important for the incidence of traumatic cutaneous myiasis, and the risk of infestation was, therefore, highest in animals from farms with old and poorly maintained facilities.

Our data also show that the presence of traumatic myiasis is related to the existence of a lambing building on the farm.

This facility is used during the lambing season for protecting ewes and their offspring from both predators and adverse weather conditions. Although *W. magnifica* shows, in contrast to some calliphorids such as *Lucilia*, *Calliphora* and *Sarcophaga*, relatively low-density environmental populations, flies tend to concentrate in areas with high density of potential hosts (Ruíz-Martínez *et al.*, 1992). In this regard, lambing buildings meet several favourable conditions for sheep to be infested by *W. magnifica*, including overcrowding and the presence of lochial fluids and remains of the placenta.

Although it has been reported that sheep are the most susceptible host to wohlfahrtiosis, *W. magnifica* larvae can develop in many other hosts (Zumpt, 1965). Our results indicated that the incidence of traumatic myiasis in other farm animal species was directly related to the total number of infested sheep at the farm; hence, every infested sheep implied a risk of infestation 1.04-fold higher than in other farm animals. The infestation of other species was also related to the geoclimatic area, being significantly lower in the MM area, which showed the lowest number of positive sheep. The use of insecticide baths (dipping) was also associated with an increased risk of infestation in other species. It should be noted that when gravid flies are not able to deposit their larvae on their main host (sheep), they search for other animal species (Ruíz-Martínez *et al.*, 1992; Giangaspero *et al.*, 2011), and the epidemiological importance of sheep as a reservoir of wohlfahrtiosis for other animals as well as potential public health implications must, therefore, be considered (Hadani *et al.*, 1971).

Lesions showed a characteristic anatomical distribution, with most larvae located in the genital area in both sexes. These results agree with most previous investigations (Isimbekov & Zhumbekov, 1983; Ruíz-Martínez *et al.*, 1992; Farkas *et al.*, 1997; Giangaspero *et al.*, 2011); the urine and shifts in mucous/skin microbial communities, both producing volatile chemicals, would make these areas particularly attractive to flies (Hall, 1997; Farkas *et al.*, 2009). Infestations of the genital organs have led to reproduction disorders in sheep (Ruíz-Martínez *et al.*, 1993; Farkas & Hall, 1998) and camels (Hadani *et al.*, 1989; Valentin *et al.*, 1997). Considering the high prevalence of wohlfahrtiosis in the genitalia, it can be assumed that prior wounding is not a key predisposing factor for wohlfahrtiosis in sheep.

Our data also revealed that the second most frequently infested body region differs in both sexes and is associated with differences in their management and behaviour. In this way dominance fights between rams could result in wounds on the head/neck area that can be easily infested by flies (Sotiraki *et al.*, 2003). These results are consistent with a previous investigation where the majority (80%) of non-genitalia infestations of males were found on the head (Sotiraki *et al.*, 2005). In contrast, after genitalia, the feet, udder and ears were the sites of predilection of *W. magnifica* in ewes from SE Spain. Similarly, Aydenizöz & Dik (2008) determined that the most infested areas in ewes were the legs, anal region, udder and hoof, and, as in our study, no head infestations were recorded in ewes.

The seasonal distribution of wohlfahrtiosis in SE Spain was unimodal; active infestations were detected for 9 months (March–November), with a peak in the summer months (June–September). Our data suggest that the fly period of *W.*

*magnifica* is currently widening, because previous studies in southern Spain and other European countries reported an activity period from April to October (Ruíz-Martínez *et al.*, 1993; Sotiraki *et al.*, 2005, 2010; Tligui & Zerhouni, 2010). This variation could be attributed to an increase in the average temperature during the last decade or to the localization of the farms. It is worth noting that noticeable variations in the fly activity period (7–9 months) were observed when the geoclimatic area was considered. In western Europe, Ruíz-Martínez & Leclercq (1994) reported that *W. magnifica* imagos showed ecdysis at altitudes from 200 to 2900 m. In this regard, the altitude can be considered as an indirect measure of specific climatic variables such as temperature, which is an important determinant of fly abundance (Wall *et al.*, 1992). French *et al.* (1994) indicated that *L. sericata* populations decrease as altitude increases, also showing a shorter duration of the flying season. Our results agree with these studies because the area with a longer activity period of flies was the MM (9 months) area, followed by the NP (8 months) and CM (7 months) areas, with a mean altitude of 685.15, 703.35 and 953.33 m, respectively.

Because traumatic cutaneous myiasis by *W. magnifica* is widespread in SE Spain, preventive measures for minimizing the attractiveness of sheep to gravid flies are necessary to avoid health and welfare problems as well as significant production losses in sheep flocks. Special attention should be paid to rams, whose wounds must not be left unattended.

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## Conflict of interest

The authors declare that there is no conflict of interest.

## Data availability statement

Data are available from the corresponding author under reasonable request.

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