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Prevalence of *Oestrus ovis* in small ruminants from the eastern Iberian Peninsula. A long-term study

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Abstract

Oestrus ovis is an obligate parasite that causes myiasis in domestic ruminants, being commonly found in the Mediterranean area. From 2009 to 2019 a total of 3476 heads of culling sheep and goats from the Mediterranean coast of Spain were examined for the presence of *O. ovis*. The total prevalence was 56.3%, significantly higher in sheep than in goats (61.2% and 43%, respectively). Differences were found in the mean annual prevalence, with the highest value being registered in 2018 (61.7%) and the lowest in 2012 (50.3%). Autumn, for sheep, and winter, for goats, were the seasons with the highest number of infested specimens. Temperature, but not rainfall, was found to be associated with prevalence ($p < 0.05$). Most L1 were found in the anatomic region I (septum, meatus, and ventral conchae), while L2 and L3 were mainly located in regions II (nasopharynx, ethmoid labyrinth, and dorsal conchae), and III (sinuses). The overall intensity was 12.8 larvae per head, significantly higher in sheep (13.3) than in goats (3.5). Our results confirm the high prevalence of *O. ovis* in sheep and goats in this geographic area over the last decade, with the trend increasing in recent years in association with higher mean temperatures.

KEYWORDS
epidemiology, Iberian Peninsula, oestrosis, *Oestrus ovis*, small ruminants

INTRODUCTION

Oestrus ovis (Linneo, 1761) (Diptera, Oestridae), also known as the sheep botfly, is an obligate parasite whose larvae cause cavitary myiasis in small ruminants (Alcaide et al., 2005; Uslu and Dik, 2006b; Dönmez et al., 2006; Angulo-Valadez et al., 2010; Attia et al., 2019). Oestrosis mainly affects sheep and goats, but accidental nasal, pharyngeal and ocular infestations have been reported in other species such as dogs (*Canis lupus familiaris*) (Zanzani et al., 2016) and in humans, typically in shepherds and farmers (Hartmannová et al., 2020). Larval migration causes constant irritation, and difficulty breathing and feeding (Dorchies et al., 1998). Although mortality is low, oestrosis affects long-term productivity and can cause major economic losses (da Silva et al., 2012; Dorchies et al., 2000; Kouam et al., 2014; Ortega-Muñoz et al., 2019). Moreover, stress caused by the flight of the adults and the presence of the larvae makes animals susceptible to other diseases (Caracappa et al., 2000; Dorchies et al., 2000; Gracia et al., 2019). Prevalence rates and larval burden are usually higher in sheep than in goats (Alem et al., 2010; Dorchies et al., 2000; Papadopoulos et al., 2010). Moreover, lesions and clinical signs are more severe in sheep, which has been attributed to the inadaptation of the parasite to goats and to a higher inflammatory response in sheep (Angulo-Valadez et al., 2010; Dorchies et al., 1998; Nguyen et al., 1999).

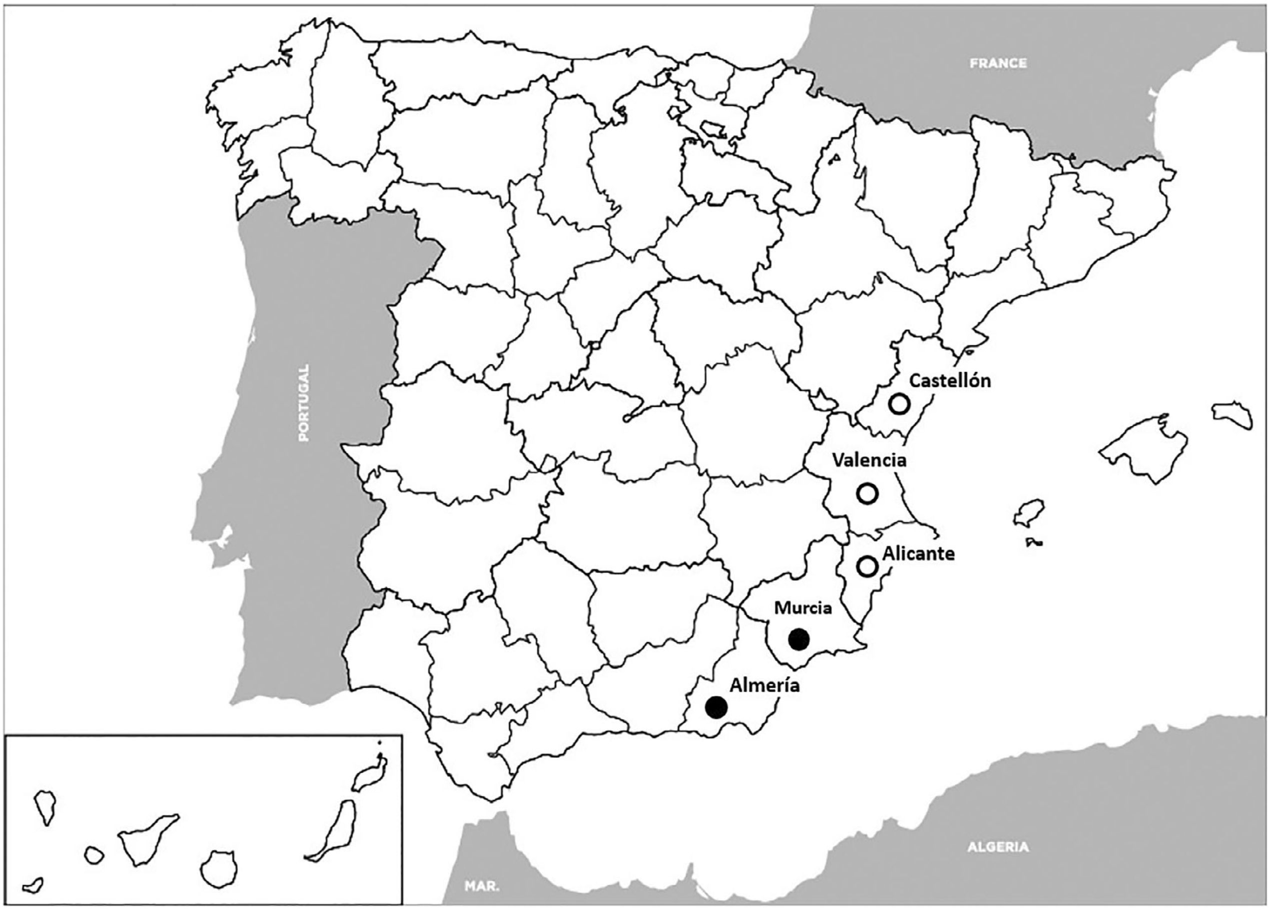

FIGURE 1 Geographical regions of the studied area, highlighting Murcia provinces (black spots) and Valencia provinces (white spots)

This parasitic disease is distributed worldwide but is especially common in Mediterranean countries, Asia, and Africa, with prevalence in sheep reaching up to 99% in some countries (Ahaduzzaman, 2019). *Oestrus ovis* can adapt to different environments (Ortega-Muñoz et al., 2019), and a wide range of relative humidity, although it favours high temperatures and solar irradiance (Cepeda-Palacios et al., 2011): up to four generations of flies may be spawned in these conditions each year (Yilma & Dorchies, 1991). Adult females deposit first-stage larvae (L1) directly into the nostril of the host, which then migrate from the ethmoturbinates to the ethmoid sinus, transforming into second-stage larvae (L2) while feeding on mucus, and finally they move on to the frontal and maxillary sinuses for the third stage (L3) (Zumpt, 1965). The timing of the sheep botfly lifecycle varies according to the season and the climatic conditions, ranging from weeks to several months, and it has been demonstrated that L1 can overwinter in a diapause state (Yilma & Dorchies, 1991). In southern Mediterranean countries, this hypobiosis period is shorter and in humid tropical countries, fly activity and larval development can occur all year-round (Tabouret et al., 2001).

During the 20th century, the world's climate warmed by 0.6°C and Europe's by 0.8°C (IPCC, 2001; Jones et al., 1999). A similar trend has been observed in Spain in recent years (AEMET, 2022). As many authors have pointed out, climatic parameters directly influence biodiversity (Arneth et al., 2020; Cavicchioli et al., 2019; Varga et al., 2019) and it has been well documented that such climatic change favours the lifecycles of arthropods such as the Diptera (Semenza & Suk, 2018). Thus, the distribution and prevalence of *O. ovis* may have changed in some geographical areas. Although many surveys have been conducted to determine the prevalence of this parasite worldwide, information continues to be lacking regarding the trend of this parasite in countries where it is endemic and long-term studies are scarce in the literature. The present work aims to contribute to the knowledge of the epidemiology of *O. ovis* in domestic small ruminants in the western Mediterranean area over the last decade within the current context of global warming.

MATERIAL AND METHODS

Animals and area of study

From February 2009 to July 2019, a total of 3476 animals, 2538 culling sheep, and 938 culling goats ranging between 6 and 10 years were examined. The animals came from two regions of Spain, comprising five coastal provinces located in the western Mediterranean: the Murcia and Almería region and the Valencia region (including the

TABLE 1 Mean annual temperature and rainfall in the two areas of study, from 2009 to 2019

	Murcia and Almería region		Valencian community	
Year	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)
2009	18.5	307.8	17.9	353.7
2010	17.9	389.8	17.6	327.3
2011	18.9	212.6	18.4	302.5
2012	18.5	220.2	18.2	220.6
2013	18.9	186.0	18.5	227.3
2014	19.6	146.8	19.1	244.0
2015	19.5	196.0	18.8	366.5
2016	19.5	299.5	18.7	390.6
2017	19.4	176.5	18.5	324.9
2018	19.1	188.5	18.5	395.9
2019	19.0	251.5	18.3	515.3

Source: www.aemet.es.

provinces of Alicante, Valencia, and Castellón). The area is located between coordinates 36° 50′ and 39° 59′ N and 2° 28′ and 0° 2′ W (Figure 1). The climate is characterized by hot and dry summers and mild winters, with irregular rainfall, concentrated in spring and autumn. Sheep and goat semi-intensive production system predominates, small ruminants representing an important sector for the Spanish food industry. During the years of the survey (2009 to 2019), the annual average temperature in the studied area was 18.7°C and the average annual rainfall was 283.76 mm (AEMET, 2022) (Table 1).

Parasitological methods

The animals from the provinces of Almería and Murcia were slaughtered in accordance with current national and European legislation in a local abattoir in Cartagena (Murcia) (Real Decreto 54/1995, Directiva 93/119/CE). The heads were placed in individual plastic bags after being separated from the body, identified, and immediately transported in a cool box with ice to the necropsy room of the Faculty of Veterinary Medicine at the Universidad de Murcia (Murcia) for a complete and exhaustive examination. The animals from the Valencia, Alicante, and Castellón provinces were euthanized (as approved by the university's ethics committee; 86/07-CEEA) in the necropsy room at the Faculty of Veterinary Medicine at the Universidad CEU Cardenal Herrera (Valencia) with sodium pentobarbital and the heads were then separated from the carcasses. All heads were cut open along their longitudinal and sagittal axis from the nose to the base with an electric saw. Three anatomical regions (I, II, and III) for larvae location were differentiated. Region I corresponded to the nasal septum, nasal meatus, and ventral nasal conchae. Region II corresponded to the nasopharynx, ethmoidal labyrinth, and dorsal nasal conchae, and region III corresponded to the paranasal sinuses (Garijo et al., 2005).

Both sinuses and nasal conchae were carefully opened for observation. The sinus bones were removed with a hammer and chisel. The brain and the ethmoidal bone were examined for the presence of larvae o lesions compatible with oestrosis. The prevalence and the total and mean abundance (±SD) of the larvae were calculated (Bush et al., 1997). The number, location, stage, and survival of larvae, and the presence of macroscopic lesions were also recorded. The larvae were washed in physiological saline and preserved in 70% alcohol until their identification, which was carried out in accordance with Zumpt (1965).

Histopathological study

Formalin 10% fixed samples from 128 positive sheep chosen at random between the most intensely infested animals (43 from region I, 48 from region II, and 37 from region III) were embedded in paraffin and three-micron-thick sections were made and stained with haematoxylin and eosin (HE). The presence of oedema, congestion, necrosis, and haemorrhage, the epithelial status, the predominant inflammatory cells, connective tissue activity, and mucous secretions were all evaluated and recorded.

Statistical analysis

The association between the presence of larvae and categorical factors was compared using Pearson's χ^2 test and the confidence intervals for prevalence estimates were calculated using the Wilson score interval. Variables were compared with Pearson's chi-squared test and Fisher's exact test. The Shapiro–Wilk test for normality and Levene's test for homoscedasticity were used to detect significant differences among group variances. Quantitative factors were analysed with a non-parametric test (Wilcoxon-Mann–Whitney test) to assess the difference between categories. Statistical analysis was performed using the R statistical software (version 3.6.1) and the Rcmdr package, freely available on CRAN. Statistical significance was set at a $p < 0.05$.

RESULTS

Out of 3476 samples, 1957 (56.3%) tested positive for the presence of *Oestrus ovis* larvae, with a higher proportion in sheep than in goats (61.2% vs. 43% respectively, $p < 0.01$). Similar total prevalences were recorded in the two geographical regions studied (53.6% in the Murcia region and 59.7% in Valencian Community) ($p > 0.05$). However, significant differences were found in annual mean prevalence across the period studied, with the highest value being registered in 2018 (61.7%) and the lowest in 2012 (50.3%) ($p < 0.05$) (OR = 0.7649; 95%, CI = 0.6131, 0.9542) (Figure 2a). A significant association was found between prevalence and temperature, but not between prevalence and rainfall ($p < 0.05$). *Oestrus ovis* larvae were observed in both

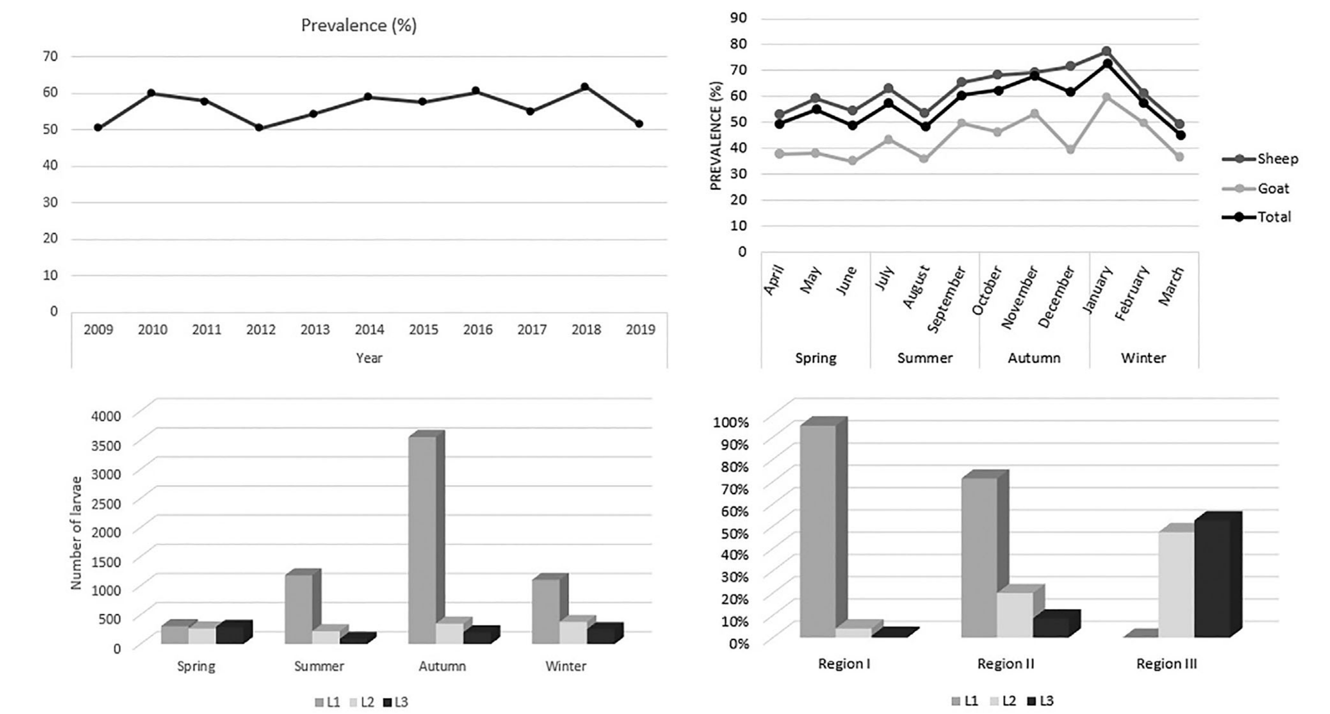

FIGURE 2 (a) Annual total prevalence of *Oestrus ovis* in sheep and goats between 2009 and 2019, (b) monthly prevalence (%) of *O. ovis* in sheep, goats, and overall, between 2009 and 2019, (c) mean number of L1, L2 and L3 of *O. ovis* in each season throughout the period of study, (d) percentages of L1, L2 and L3 of *O. ovis* in the three anatomical regions examined.

host species in each month of every year. Seasonal variation was found, prevalence was highest in autumn for sheep (69.8%) and in winter for goats (48.4%) ($p < 0.05$). January was the month with the highest rate in both sheep (77.3%) and goats (59.6%), followed by December in sheep (71.6%) and November in goats (53.3%). The lowest percentages were registered in March for sheep (49.3%) and in June for goats (34.6%) (Figure 2b).

A total of 24,984 larvae were collected, 18,442 at the L1 stage (73.8%), 3691 at L2 (14.8%), and 2851 at L3 (11.4%). The number of L1 was significantly higher than those for L2 or L3 ($p < 0.05$). In sheep, a total of 20,732 larvae were found, of which 15,554 (75%) were L1, 2949 (14.2%) L2, and 2229 (10.8%) L3. In goats, the total number of larvae found was 4252: 2888 L1 (67.9%), 742 L2 (17.5%), and 622 L3 (14.6%). Most of the larvae (98.5%) were alive. Of the 404 dead larvae, 215 (53.2%) were L3, 121 (30%) L2 and 68 (16.8%) L1. Autumn was the season in which the most L1 were recovered ($p < 0.05$), although a peak of 3978 larvae was recorded in December, while L2 and L3 were most abundant in winter and spring (Figure 2c).

The mean total larvae per head was 12.7 ± 9.5, with a significantly higher ($p < 0.05$) intensity for L1 (9.4 ± 14.9 larvae/head; min: 1, max: 125) than L2 (1.9 ± 1.8 larvae/head; min: 1, max: 10) or L3 (1.5 ± 1.4 larvae/head; min: 1, max: 10). The mean total larval intensity in sheep was higher than in goats (13.3 ± 9.7 larvae/head vs. 3.5 ± 8.6 larvae/head, respectively, $p < 0.05$). In sheep, the mean intensity for each larval stage was: L1, 6.1 ± 16; L2, 1.2 ± 1.8; and L3, 0.9 ± 1.4. In goats, the means recorded were: L1, 3.1 ± 15.5; L2, 0.8 ± 1.8; and L3, 0.7 ± 1.9.

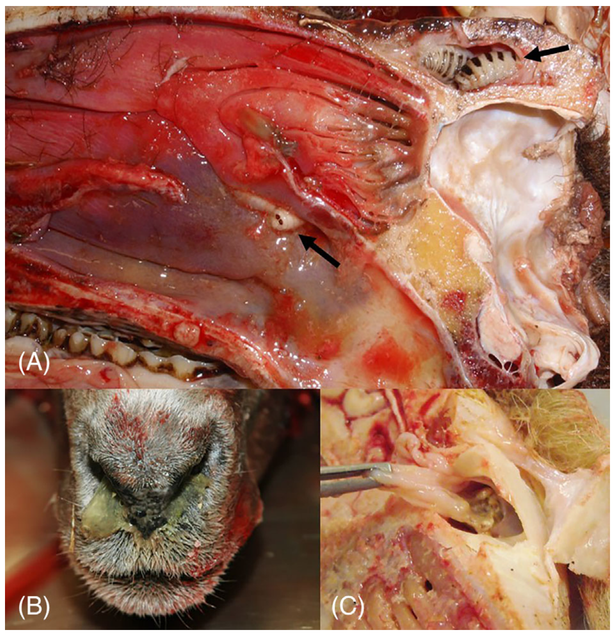

FIGURE 3 Sheep infested with *Oestrus ovis*. (a) Hyperaemia, mucous exudate, and two L2 in the conchae mucosa and two L3 in the frontal sinus, (b) seromucous nasal discharge, (c) sinusitis in the frontal sinus with remnants of dead larvae.

Of the total larvae, 58.2% were found in region I, 25.3% in region II, and 16.5% in region III. In regions I and II, L1 predominated among the other two stages (95.1% and 78.1% respectively, $p < 0.05$),

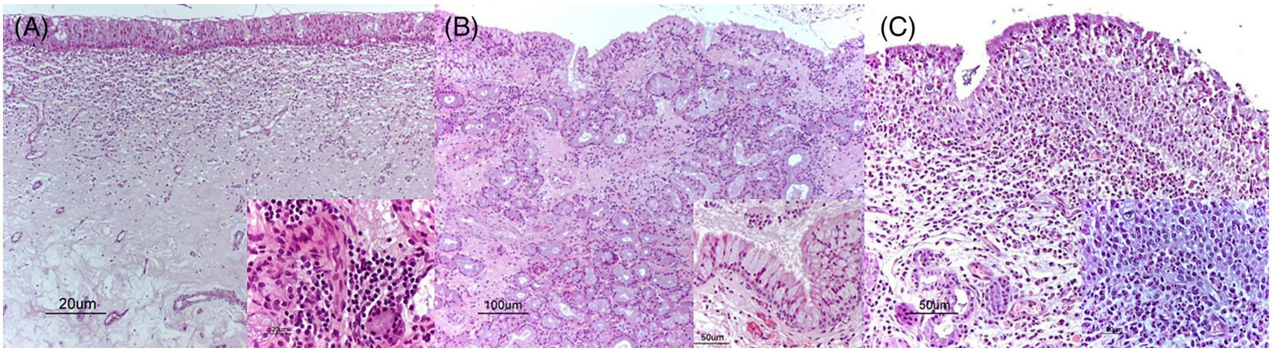

FIGURE 4 Histological images from regions II and III in an infested sheep. (a) Frontal sinus (region III), severe superficial oedema with lymphoplasmacytic and eosinophilic infiltrate (in detail), (b) nasal cavity (region II), hyperplasia of submucosal glands with inflammatory interstitial infiltrate. In detail: marked hyperplasia of goblet cells in pseudostratified ciliated columnar epithelium, (c) nasal cavity (region II): severe eosinophilic infiltrate in the submucosa (in detail), with multifocal areas of exocytosis (intraepithelial migration).

TABLE 2 Histological findings in samples from infested sheep with *Oestrus ovis*

	No. samples examined	Oedema +(%)	Lymphoplasmacytic infiltration +(%)	Eosinophils +(%)	Hyperplasia +(%)	Epithelial necrosis +(%)
Region I	43	35 (81.4)	40 (93%)	5 (11.6)	10 (23.3)	0 (0)
Region II	48	48 (100)	45 (93.8)	6 (12.5)	25 (52.1)	0 (0)
Region III	37	37 (100)	30 (81.1)	4 (51.4)	19 (51.4)	3 (8.1)

whereas in region III stages L2 and L3 were more abundant (48.5% and 51.3%, respectively) (Figures 2d and 3).

Macroscopically, 54.7% of the positive animals showed hyperaemia in one or more of the three regions. Mucous discharge was observed in 48.4% of the animals and sinusitis in 29.7%, although no significant differences were found between the host species (Figure 3). Four animals had an abscess containing dead larvae in the frontal sinus. In 81.1% of the animals showing macroscopic lesions, larvae of one or two instars were found and, in the remaining 18.9%, larvae from the three instars were recovered. No larvae or lesions were found in the brain or the ethmoidal bone in any animal.

The histological study showed different grades of inflammation in all the examined samples from the nasal cavity and paranasal sinuses (Figure 4, Table 2). In 89.8% of cases, chronic lymphoplasmacytic infiltration was observed. Nasal epithelium goblet cells, hyperplasia of the submucosal glands, and oedema were also frequent in samples from the three anatomical regions. To a lesser extent, eosinophils were also found. Epithelial necrosis was detected only in samples from region III.

DISCUSSION

This study tracks the prevalence of oestrosis, an economically important and endemic parasitic infection, in sheep and goats in the Mediterranean coast of the eastern Iberian Peninsula, for over 10 years. The total mean prevalence (56.3%) found was lower than that observed in previous similar studies from the eastern area of Spain, such as the mid-Ebro Valley (84.2%) (Gracia et al., 2010) or the Balearic Islands (84%) (Paredes-Esquivel et al., 2009), but higher than that reported in this latter region some years later by the same authors (46%) (Paredes-Esquivel et al., 2012). In other surveys, values range between 11.5% and 67.4% in Mediterranean countries such as Italy, France, Algeria, Turkey, Greece, Egypt, and Libya (see Ahaduzzaman, 2019).

No significant differences were found between the two geographical regions studied here, which was not unexpected due to their proximity and the similarity in the mean annual temperature and rainfall throughout the period of the study.

The highest prevalence values were observed in autumn in sheep and winter in goats. The highest quantities of L1 were recovered during the summer and autumn months, while L2 and L3 were more abundant in winter and spring, which suggests higher botfly activity during spring and summer and is consistent with previous studies in the same geographical area (Caracappa et al., 2000; Paredes-Esquivel et al., 2012). In most studies carried out in the Mediterranean, the prevalence of *O. ovis* has been found to be associated with climatic conditions, in contrast with findings from other areas with similar temperatures and rainfall during the year, such as Brazil (Da Silva et al., 2012). This received further confirmation in the recent meta-analysis of data from around the world by Ahaduzzaman (2019), in which the effect of climatic conditions on oestrosis was analysed. As is well known, larviposition usually occurs on warm and sunny days, at temperatures greater than 20°C and the larvae present the greatest level of activity at 22–28°C (Angulo-Valadez et al., 2010; Cepeda-Palacios et al., 2011). Larval development can take between 3 weeks and several months, depending on the geographical region (Dorchies et al., 2000; Yilma & Dorchies, 1991). In the Mediterranean area, the average annual temperature is around 18–19°C, with peaks of over 22°C in spring and summer (AEMET, 2022), which are the seasons with higher adult fly activity, and thus larvae are found in greater abundance during the colder seasons. Nevertheless, given that we found the three larval stages in both host species in each month of every year, our results suggest the existence of a long favourable period for larval development, with perhaps three or four generations of flies throughout the year in the area of study, as has been previously proposed (Yilma & Dorchies, 1991). The presence of L3 throughout the year has also been observed in other surveys (Gracia et al., 2010; Papadopoulos et al., 2010): it may be that there is an absence of inhibition for larval development and L3 may also remain unexpelled inside the host (Gracia et al., 2010, 2019), which could also explain our findings.

The annual mean prevalence in this study showed statistical differences for 2012, in which the percentage of infested animals was significantly lower than other years. This could be attributed, in part, to the fact that mean temperatures and rainfall during 2011 and 2012 were low, meaning that climatic conditions were less favourable for the development of the cycle. Another reason could be the use of effective endectocides on the animals against *O. ovis* to prevent nematodosis, or after high infestation periods, when animals show severe clinical signs of oestrosis. In previous studies, a decrease in prevalence was observed after the treatment of the flock with macrocyclic lactones (Da Silva et al., 2012) or closantel (Jacquiet & Dorchies, 2002). Although not significant, a trend towards higher prevalence of oestrosis was observed in the final years in the area of study. Furthermore, the percentage of positive animals is higher in the present study when compared with the one conducted in Murcia from 1998 to 2001, one of the provinces included in our survey, which reported that 38.1% of sheep were infested with *O. ovis* (Garijo et al., 2005). Climatic conditions may be the main reason for this trend, especially temperature, which has increased in recent years in this area and showed a significant relationship with prevalence in the present survey (AEMET, 2022).

The mean larval intensity was similar to other studies carried out in Spain (Paredes-Esquivel et al., 2009, 2012), although lower than the value found by Gracia et al. in 2010 in the mid-Ebro Valley. In Murcia, one of the provinces included in this study, Garijo et al. (2005) found a higher larval burden in adult sheep (23.2 larvae/head). This difference may be attributed to the fact that in the last year of this latter study, samples were only taken for 6 months, including January, the one with the highest mean larval burden of the year.

Regarding the host species, both prevalence and larval burden were higher in sheep than in goats, which is consistent with the surveys undertaken in France, Greece, and countries in Africa (Dorchies et al., 2000; Gebremedhin, 2011; Papadopoulos et al., 2001; Papadopoulos et al., 2006). These differences between species may be explained by the inadaptation of *O. ovis* to goats and the higher effectiveness of goats in avoiding larviposition (Dorchies et al., 1998). Some authors also suggest a relationship between the lower prevalence in goats and their lower sensitivity to fly-strikes, which could be attributed to their browsing habits (Hoste et al., 2001). However, goats may represent a problem for the control of oestrosis, since farmers do not treat them in the absence of severe clinical signs and thus goats may act as a reservoir species for the parasite (Jacquiet & Dorchies, 2002).

Age has been shown to be a risk factor for infection. Older animals are more likely to be infected in most cases (Abo-Shehada et al., 2003; Papadopoulos et al., 2010), even if there have been reports to the contrary (Gebremedhin, 2011). It has been suggested that immunosuppression occurs after repetitive reinfections (Jacquiet et al., 2005), which could explain an increase in prevalence in older hosts.

In addition to geographical area, age, and host species, there are other risk factors for the presence of oestrosis, such as poor body condition (Gebremedhin, 2011). Different age groups could not be compared in this study since all the sheep and goats examined in this survey were old. However, they were also all affected by other concomitant parasitic and infectious pathologies, which may have predisposed them to infection with *O. ovis*. Another factor linked to differences in prevalence is the diagnosis technique employed, as demonstrated in studies focusing on the south-west of Spain (Alcaide et al., 2005; Angulo-Valadez et al., 2008). Serological methods are usually more sensitive than visual inspection, with ELISA being the procedure of choice for detection in vivo., but false positives may skew such data. Although both visual and serological methods are currently employed, necropsy is more reliable than either (Attia et al., 2019). Recent studies have assessed the diagnostic usefulness of semi-nested PCR and rhinoscopy (Ípek & Altan, 2017) and the analysis of the cell-mediated immune response by qRT-PCR (Attia et al., 2020), but these are expensive techniques for use in fieldwork on small ruminants.

As with earlier reports from the Mediterranean coast, L1 larvae were the most abundant (Alcaide et al., 2005; Uslu & Dik, 2006a; Paredes-Esquivel et al., 2009). Rogers and Knapp (1973) estimated that approximately only 8% of L1 develop into L2, due to the response of the host immune system to myiasis (Angulo-Valadez et al., 2008, 2011) and, as mentioned above, in this study all the animals were old and probably immunocompromised. However, the proportion of L2 and L3 recorded was also high when compared to previous Mediterranean reports, probably due to the mean annual temperatures in the area of study, which may favour the development of L3 (Yilma & Dorchies, 1991). Regarding distribution, most L1 larvae were in the anatomical region I, whereas L2 and L3 were mostly found in regions II and III, corresponding to the anatomical areas where the two moults take place, in accordance with previous findings (Garijo et al., 2005; Yilma & Dorchies, 1991). These same authors observed a high number of dead L3 larvae in the sinuses of region III, which can be explained by an increase in size that impedes them to return to nasal cavity until they finally die (Garijo et al., 2005). In some of these cases, it is not unusual for abscesses to occur; in this study, abscesses were found in four animals in the frontal sinuses.

The macroscopic lesions observed were similar to those previously reported, and were associated with the presence of both live and dead L2 and L3 larvae (Garijo et al., 2005; Saleem et al., 2017; Yilma & Dorchies, 1991). Gebremedhin (2011) found that nasal and sinus cavity pathology was positively correlated with the total larval count. On the other hand, some authors have argued that lesions derive from a hypersensitive reaction from the host rather than trauma effected by the larvae, and so larval viability would not be a determinant factor (Abo-Shehada et al., 2003; Angulo-Valadez et al., 2010; Dorchies et al., 1998).

Following Angulo-Valadez et al. (2008, 2011), we found no relationship between larval survival and development, on the one hand, and a local inflammatory response, on the other. Histologically, all samples showed inflammation. An elevated number of lymphocytes and plasmatic cells have also been observed by Nguyen et al. (1999) and Tabouret et al. (2003). The lower values of eosinophils which we found are not consistent with those reported elsewhere, probably because most of the examined animals presented reinfestations, as high concentrations of eosinophils usually occur after the first and the second contact with *O. ovis* (Angulo-Valadez et al., 2010; Nguyen et al., 1999).

The present long-term study shows a high prevalence of loss-generating myiasis in small ruminants from the Iberian Peninsula, especially sheep. The zoonotic potential must also be considered since accidental ophthalmomyiasis is not infrequent, and sometimes results in a total loss of vision (Pampiglione et al., 1997). Although the ideal control measure would be to protect animals against fly attack, this is almost impossible under field conditions. Since clinical manifestations are closely related to climatological conditions and during L1 hypobiosis there are no clinical signs, it would be of great interest to adapt control measures suited to the climatic conditions of each geographical region, using a One Health approach (Angulo-Valadez et al., 2010, 2011). Finally, given that climate warming favours the lifecycles of Diptera (Semenza & Suk, 2018), it is to be expected that reinfection will become more common, making more epidemiological surveys on *O. ovis* in small ruminants necessary, particularly in Mediterranean areas, so that this parasitosis can be monitored and counteracted more effectively.

AUTHOR CONTRIBUTIONS

Conceptualization: María Magdalena Garijo-Toledo, and María Rocío Ruiz de Ybáñez-Carnero; Methodology: María Magdalena Garijo-Toledo, María Rocío Ruiz de Ybáñez-Carnero, José Sansano, Francisco Domingo Alonso de Vega, and Carlos Martínez-Carrasco; Statistical analysis: José Sansano and Lola Llobat; Writing-original draft preparation: María Magdalena Garijo-Toledo; Writing-review and editing: María Magdalena Garijo-Toledo and Ana Elena Ahuir-Baraja. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Data openly available in a public repository that does not issue DOIs

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