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Prevalence of *Oestrus ovis* Larval Infestation in the Slaughtered Sheep Population of Sardasht County, West Azerbaijan, Iran

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Abstract Oestrus ovis, commonly known as the nasal botfly, has a global distribution and causes myiasis in sheep and goats. The present study aimed to evaluate the prevalence of Oestrus ovis larval infestation in the slaughtered sheep population of Sardasht County, West Azerbaijan, Iran. This cross-sectional study was conducted over a 12-month period at the local slaughterhouse. Each month, 34 sheep were examined, and data regarding gender, age, breed, and sampling time were analyzed. Among the 408 sheep examined, 138 cases (33.8%) were found positive for infestation. The highest prevalence was observed in sheep older than three years (16.7%), while the lowest was recorded in sheep younger than one year (2.4%), indicating a significant correlation between infestation rate and age (P < 0.05). The highest prevalence was observed in summer (11.0%) and the lowest in winter (5.9%). The month-wise analysis showed the peak infestation in August (3.9%) and the lowest in February (1.7%), with a statistically significant seasonal pattern (P < 0.05). However, no significant association was found between infestation rate and sheep gender or breed (P > 0.05). This study highlights Oestrus ovis as a prevalent parasite in Sardasht County, capable of causing substantial economic losses in sheep farming. Additionally, due to its zoonotic potential, the parasite poses a public health concern.

Introduction

Sheep and goat farming, characterized by low investment and rapid economic returns, serves as a critical livelihood resource in low-income regions [1]. These animals efficiently convert marginal lands into valuable outputs such as milk, meat, and by-products, supporting nearly one billion head globally and contributing significantly to household sustenance and economic stability. Considering the critical role of sheep and goat farming, effective management and maintaining optimal health are essential to ensure productivity and sustainability [2]. These animals are particularly susceptible to various health challenges, including viral [3], parasitic [4, 5], bacterial [6], and metabolic diseases [7], which pose significant threats to their well-being and productivity. A wealth of research has been conducted to address these challenges and develop effective prevention and management strategies [3].

The parasite *Oestrus ovis*, commonly known as the nasal botfly [8], belongs to the family

Oestridae and is responsible for causing myiasis in sheep and goats [9]. This parasite has a global distribution, with higher prevalence observed in warm and humid regions [10]. The adult botfly exhibits a flight behavior similar to that of honeybees, with an average lifespan of up to 10 days. It quickly approaches the noses of sheep to deposit its eggs. Female botflies lay larvae in their first instar stage (L1) at the nasal openings, leading to the development of rhinitis [11]. The larvae migrate to the frontal sinuses, where they grow into the L2 and L3 stages, feeding on mucosal secretions, which can lead to the development of sinusitis [12]. Upon reaching the L3 stage, these larvae return to the nasal passages, are expelled through sneezing, and complete their development on the ground until they reach maturity [13]. Notably, larvae identified at the L1 stage have been observed to cause zoonotic diseases, representing a potential health risk for both livestock and humans [14]. Common signs include nasal discharge, sneezing, head shaking, labored breathing, restlessness, and in severe cases, reduced feed intake and weight loss. These symptoms are caused by the irritation and inflammation of the nasal passages and sinuses due to larval activity [15]. This highlights the significant veterinary and public health challenges associated with Oestrus ovis infestations [16, 17].

The aim of this study is to evaluate the prevalence of *Oestrus ovis* larval infestation in the slaughtered sheep population of Sardasht County, West Azerbaijan, Iran. The research seeks to identify the relationship between infestation rates and various factors such as age, gender, breed, and seasonal variations, while also highlighting the parasite's potential economic and public health implications.

Materials and methods

Sardasht County, situated in the southwestern region of West Azerbaijan province, Iran, spans an area of 1,411 km². Geographically, it is located at a latitude of 36°9′ N and a longitude of 45°28′ E, with an elevation of 1,510 meters above sea level. The region experiences an average annual temperature of 14.01°C, with annual precipitation averaging 926.8 mm. In 2019, the area recorded up to 101 days of cold and freezing conditions [18].

Study population

In the present study, the statistical population was determined using Epi Info software (version 7.2.5.0) to ensure accuracy and reliability in calculations. Since, the exact population of sheep in Sardasht County is not officially documented, an estimated population of 400,000 was considered based on available regional livestock data [19]. Previous studies have reported a prevalence range of 30% to 58% for Oestrus ovis infestation in similar populations. Therefore, by selecting a prevalence rate of 32%, a representative and reasonable estimate derived from existing literature, the statistical sample was calculated, resulting in a total of 408 sheep being included in this study. This estimation allows for a practical and evidence-based approach to assessing the target population while accounting for potential variations in the prevalence rates reported across prior research. In the present study, a total of 408 sheep were examined, comprising 193 rams and 215 ewes. The subjects were categorized into four age groups: under one year, one to two years, two to three years, and over three years. Additionally, the sheep were classified into four breed categories for analysis: Ghezel, Kurdish, Afshari, and other breeds. The study was conducted over a 12-month period to ensure comprehensive data collection across seasonal variations.

Sample collection and examination procedure

The present study was conducted from May 2020 to April 2021. Livestock slaughterhouses were systematically visited to collect the required samples. Initially, all selected animals were marked to ensure proper identification, allowing for efficient tracking of sample numbers and corresponding data following slaughter. Relevant information, including age, sex, date, and breed of each sheep, was recorded on standardized data collection forms during this stage.

The age of the animals was estimated through a combination of methods. Prior to slaughter, information about the animals, such as records provided by the farm or the handlers, was collected to determine their approximate age. After slaughter, a dental inspection was conducted, which is a standard method for accurately estimating the age of livestock. This approach involves examining the eruption and wear patterns of the teeth, providing a reliable estimate of the animal's age. This dual-method ensured greater accuracy approach in categorizing the animals into the predefined age groups for the study.

After the animals were slaughtered, each sample was meticulously examined for the presence of *Oestrus ovis* larvae within the nasal cavity. The detection of larvae was considered evidence of a positive sample. Using sterile forceps, larvae were carefully extracted and transferred into a container containing formalin for preservation. The preserved samples were subsequently transported to the Parasitology Laboratory at the Islamic Azad University, Urmia branch, West Azerbaijan, Iran, for comprehensive parasitological analysis [17, 20].

Statistical analysis

The analysis of data obtained from laboratory results and clinical findings was performed using SPSS (version 19) and Excel (version 2013) softwares. To assess the relationships between variables, the chi-square test (χ^2) was employed. Statistical significance was defined as P < 0.05, in accordance with standard statistical analysis guidelines.

Results

The absolute and relative frequency of infection was statistically analyzed in relation to the age of the animals (Table 1). According to the output of statistical software, among the 76 lambs (under one year of age), 10 were found to be positive, representing 2.5% of the total population (408 sheep). In the 1 to 2-year age group, 24 positive

cases were recorded, accounting for 5.9%, while in the 2 to 3-year age group, 36 positive cases were identified, corresponding to 8.8%. In the group of sheep older than 3 years, 68 positive cases were observed, making up 16.7%. Statistical analysis indicates a significant relationship between age and the prevalence of infection in the studied sheep population (p<0.05). Further analysis was conducted to assess the relationship between infection rates and the sex of the animals (Table 1). Among 193 rams, 70 were found to be positive, representing 17.2% of the total, while 68 out of 215 ewes positive, corresponding to 16.7%. tested Statistical analysis revealed no significant relationship between infection rates and the sex of the animals (p>0.05). The relationship between infection rates and the breed of the animals was also evaluated (Table 1). Among the studied population, 127 sheep belonged to the Afshari breed, with 43 testing positive, representing 10.5%. From the Kurdish breed (86 sheep), 30 were positive, corresponding to 7.0%. Among the Ghezel breed (83 sheep), 26 cases were positive, accounting for 6.4%, while 112 sheep from other breeds had 39 positive cases, corresponding to 9.6%. Statistical analysis revealed no significant association between infection rates and breed categories (p>0.05).

The prevalence of infection was also analyzed across different seasons of the year (Table 2). In the spring season, 40 out of 102 sheep tested positive, representing 9.8%. During summer, 45 positive cases were observed among the studied population, corresponding to 11.0%. In the autumn season, 29 positive cases were identified, accounting for 7.1%, while in winter, 24 positive cases were recorded, corresponding to 9.5%. Statistical analysis revealed a significant association between the infection rate and seasonal variations (p<0.05). Table 2 presents the infection rates across different months and corresponding seasons during the sampling period. A total of 34 samples were collected each month (Table 2, Figure 1). The highest prevalence of infection was observed in August. with 9.3%, while the lowest was recorded in

Age	Positive cases (%)	Negative cases (%)	P value
Younger than 1 year	10 (2.4)	66 (16.2)	
1 to 2 years	24 (5.9)	71 (17.4)	
2 to 3 years	36 (8.8)	78 (19.1)	0.007
Older than 3 years	68 (16.7)	55 (13.5)	
Total	138 (33.8)	270 (66.2)	
Gender	Positive cases (%)	Negative cases (%)	P value
Male	70 (17.1)	123 (31.1)	
Female	68 (16.7)	147 (36.1)	0.385
Total	138 (33.8)	270 (66.2)	
Breed	Positive cases (%)	Negative cases (%)	P value
Afshari	43 (10.5)	84 (20.6)	
Kurdish	30 (7.3)	56 (13.7)	
Ghezel	26 (6.4)	57 (14.0)	0.471
Other	39 (9.6)	73 (17.9)	
Total	138 (33.8)	270 (66.2)	

Table 1. Comparison of infection prevalence by age, sex and breed in the slaughtered sheep population of Sardasht county, West Azerbaijan, Iran

Table 2. Comparison of infection frequency in the slaughtered sheep population by season and months of the year in

 Sardasht county, West Azerbaijan, Iran

Season	Positive cases (%)	Negative cases (%)	P value
Spring	40 (9.8)	62 (15.2)	
Summer	45 (11.0)	57 (14.0)	
Autumn	29 (7.1)	73 (17.9)	0.023
Winter	24 (5.9)	78 (19.1)	
Total	138 (33.8)	270 (66.2)	
Months	Positive cases (%)	Negative cases (%)	P value
March	13 (3.2)	21 (5.1)	
April	13 (3.2)	21 (5.1)	
Мау	14 (3.4)	20 (4.9)	
June	15 (3.7)	19 (4.7)	
July	16 (3.9)	18 (4.4)	
August	14 (3.4)	20 (4.9)	
September	12 (2.9)	22 (5.4)	0.002
October	9 (2.2)	25 (6.1)	
November	8 (2.0)	26 (6.4)	
December	8 (2.0)	26 (6.4)	
January	7 (1.7)	27 (6.7)	
February	9 (2.2)	25 (6.1)	
Total	138 (33.8)	270 (66.2)	



Fig 1. Comparison of infection frequency in the slaughtered sheep population by season and months of the year in Sardasht county, West Azerbaijan, Iran

February, with 7.1%. Statistical analysis revealed a significant relationship between the infection rates and the months of the year (p<0.05).

Discussion

Oestrus ovis, an obligate parasite causing myiasis in sheep and goats, is also recognized as a zoonotic disease impacting both humans and animals. Its global prevalence highlights the importance of understanding and managing this parasitic infection [17].

Reports on the prevalence of this parasite in sheep populations have been documented in various countries, including Italy (55.77%) [21], Iran (49.70%) [22], Turkey (40.31%) [23], Morocco (69.17%) [24], Greece (43.15%) [25], Romania (50%) [26], Spain (45.09%) [27], Brazil (16.90%) [28], Chile (60.92%) [16], Mexico (33.24%) [29], France (43.42%) [30], Bangladesh (24.82%) [31], Germany (50.30%) [32], and Jordan (24.93%) [33]. In the present study, the prevalence of infection in Sardasht County, located in West Azerbaijan province, Iran, was found to be 33.8%. Further analysis of these studies will be discussed in detail.

In a study conducted by Caracappa et al, from May 1996 to April 1998 in Messine (Sicily), the prevalence of *Oestrus ovis* was investigated by examining 841 sheep heads from local flocks. The results showed that 55.8% of the heads were infested, with an average of 9.4 larvae per infected sheep, predominantly in the first instar stage (L1). Seasonal variation was observed, with a higher proportion of L1 larvae from October to December, suggesting slowed development. The study highlights the need for effective parasiticide use in routine parasite control to reduce the prevalence of this disease [21].

In a study conducted at the Fars abattoirs in Shiraz, southern Iran, 2002 sheep heads were examined for *Oestrus ovis* larvae. The results revealed a 49.7% infestation rate, with larvae present across all seasons, sexes, and age groups. A total of 6264 larvae were collected, with an overall intensity of 6.3 larvae per infested sheep. Seasonal prevalence ranged from 23.3% in spring to 80% in winter, with older animals showing higher infestation levels. The findings emphasize the need for targeted control measures to manage this parasitic infection [22]. In a study conducted by Tavassoli et al, in Urmia County, the prevalence of *Oestrus ovis* larvae was investigated by examining 402 sheep heads randomly selected from various breeds at the local abattoir. The results showed that 30.34% of the heads were infested, with infestation rates increasing with age and the highest prevalence observed in Ghezel sheep. Seasonal variation was minimal, with prevalence rates ranging from 27% in winter to 33.66% in autumn. The findings emphasize the need for targeted parasite control measures to manage *Oestrus ovis* infestation in this region [34].

In a study conducted by Arslan et al, in Kars province, northeastern Turkey, the prevalence of cavical myiasis caused by Oestrus ovis larvae was investigated by examining 387 sheep heads monthly over a 12-month period. The results showed that 40.3% of the sheep were infested with Oestrus ovis larvae. Seasonal prevalence was highest in spring (54.3%) and lowest in fall (28%), with statistically significant differences among the seasons (p<0.05). Infestation rates increased with age, being 30% in sheep up to 1 year old, 40% in those aged 1 to 3 years, and 52.4% in sheep older than 3 years. The mean number of larvae was 1.8 among all examined animals and 4.5 among infested animals. Furthermore, Morkaraman breed showed a higher infestation rate (43.4%) compared to the Akkaraman breed (31.3%), and sheep with darkcolored heads had higher infestation rates than those with light-colored heads (p<0.05). These findings suggest the influence of age, breed, and color on Oestrus ovis infestation rates [23].

In a study conducted to investigate the epidemiology of *Oestrus ovis*, two methods were used: a slaughterhouse survey and a tracer lamb's study. The prevalence of infection ranged from 10% to 100%, with the highest rates observed between June and September and the lowest in March. The mean annual larval burden was 6.7 larvae per sheep, with a maximum of 35 larvae recovered from a single head in September. Two distinct larval peaks were observed: the first in May and June, and the second in September and October. Flies were

absent from December to March, with the first generation of flies appearing in April. Up to four generations of flies may occur within a single year. During the hottest month of August, flies were either absent or present in negligible numbers. The study highlights that during winter, the species is maintained through overwintering larvae in the heads of sheep and pupae on the ground [24].

The findings of these studies demonstrate that variations and differing climatic weather conditions exert a direct influence on the prevalence of Oestrus ovis infection. Significant differences in infection rates across various months and seasons suggest that the temperature and humidity levels necessary for the parasite's development differ among these regions, providing a plausible explanation for the observed disparities. In the present study, the highest prevalence of Oestrus ovis was recorded during the summer months. This finding contrasts with results reported from Italy [21] but is consistent with observations from Morocco [24]. Notably, regional differences within Iran further highlight these variations; for example, the prevalence reported in Urmia County [34] differs from that in Sardasht County. The latter exhibits distinct climatic conditions, including prolonged winters due to its mountainous geography and elevated humidity levels, which are likely contributing factors to these variations.

Studies indicate that the likelihood of exposure to *Oestrus ovis* increases with the age of livestock, leading to a higher probability of infection in older animals. In the present study, the prevalence of infection was significantly higher in sheep older than three years compared to other age groups. This pattern has been consistently reported in multiple studies, supporting the association between age and increased susceptibility to *Oestrus ovis* infestation [22, 23, 34].

The impact of breed on the prevalence of *Oestrus ovis* infection has been widely investigated in various studies [28]. Arslan et al, reported that sheep with dark-colored coats are significantly more susceptible to infection compared to other sheep [23]. This finding is also supported by Carvalho's study in Brazil [28], as

well as the findings of Amarante et al. in 2004 [35], Tavassoli et al. in 2012 [34] and Bricarello in 2005 [36], who observed similar results. However, this hypothesis cannot be conclusively proven, as Silva et al. in 2012 [37] did not report significant associations between breed and infection rates. In the present study, no statistically significant relationship was observed between sheep breed and the prevalence of *Oestrus ovis* infection.

The impact of gender on the prevalence of *Oestrus ovis* infection has been reported in various studies. However, Tavassoli et al. in 2012 [34], Hidalgo et al. in 2015 [16], and Shoorijeh et al. in 2009 [22] found no statistically significant association between gender and infection rates. Similarly, the present study did not observe any statistically significant relationship between gender and the prevalence of *Oestrus ovis* infection.

Conclusion

The present study determined the prevalence of Oestrus ovis in Sardasht County, West Azerbaijan, Iran, at 33.8%. The findings revealed that multiple factors, including age, seasonal variation, and climatic conditions, significantly influence the rate of infestation. The highest prevalence was observed during the summer months, while infection rates were notably higher in sheep older than three years. However, no statistically significant association was found between Oestrus ovis prevalence and breed or sex. Climatic differences and regional weather patterns may explain variations in prevalence across different geographic areas. To effectively mitigate the spread of this parasitic infection, strategic preventive measures and targeted treatments during critical seasonal periods are strongly recommended.

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Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

The research protocol ensures that the rights, welfare, and dignity of participants are protected throughout the research process.

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