

PREVALENCE, SEASONAL DYNAMICS, AND POTENTIAL FACTORS ASSOCIATED WITH CAPRINE NASAL MYIASIS CAUSED BY *OESTRUS OVIS* IN LODHRAN, PUNJAB, PAKISTAN

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DOI: <https://doi.org/10.5281/zenodo.22702916>

Keywords

Oestrus ovis, caprine oestrosis, nasal myiasis, prevalence, seasonal dynamics, Pakistan

Article History

Received: 01 June 2026

Accepted: 23 August 2026

Published: 11 September 2026

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Abstract

Oestrosis is a parasitic disease of nasal myiasis and adjoining sinuses of sheep and goats caused by larvae from *Oestrus* genus and is a disease of economic significance in small ruminants. This study was aimed to estimate the prevalence, seasonal dynamics, age-group larval burden, and potential factors associated with infestation of caprine oestrosis. A total of 47 goat heads were collected from participating abattoirs from October 2021 to April 2022 at Lodhran district Punjab, Pakistan. Heads of goats ($n = 47$) slaughtered in Lodhran district, Punjab, Pakistan, were inspected for presence of *Oestrus ovis* larvae (L). Heads were split along longitudinal axes, and larvae (L1, L2, and L3) were collected. On analyzing the results, we found that the prevalence rate in goats was (40%; 19/47), and the prevalence rate varied from 25% in March and April-2022 to 71% in November 2021. Animals between age 1-2 years (52%) were more infected with larvae as compared to younger animal 0-1 year (18%) and animals older than 2 years. The prevalence was higher in females (42.9%) than in males (33.3%), though this difference was not statistically significant ($P = 0.736$). Out of the 53 collected larvae from goats, 0% was L1, 11% were L2, and 88% were L3 and the mean number of larvae in infected goats where: 2.79 ± 2.1 (range: 1-8). Myiasis-causing larvae collected from Lodhran, district, Punjab, Pakistan, were authenticated as *Oestrus ovis*, according to morphological characteristics. In conclusion, these findings may be used to design control strategies for myiasis in goats. In order to lower the overall prevalence of this disease, it would be necessary to use effective

pesticides and insecticides against Oestrus ovis, in all routine parasite control treatments especially in females and young animals.

INTRODUCTION

Myiasis, or infestation with fly larvae, is widespread in both domestic and wild animal species worldwide [1]. Oestrosis is a nasal myiasis 'sheep nasal fly or nasal bot fly', caused by infestation of certain larvae of flies belonging to the genus *Oestrus* (Diptera: Oestridae), which grow in nasal cavities and frontal sinuses of afflicted animals, developing from first to third stage larvae [2]. The livestock industry, which is foundation of agriculture sector, is frequently disparaged, it nevertheless contributes significantly to Pakistan economy by supplying valuable organic animal derived proteins, draught power, and various by-products (hides, bones, mohair, skin, manure and wool, etc.) [3]. According to the Economic Survey of Pakistan 2024-25, the overall number of goats increased by 2.4 million, from 87 million to 89.4 million. Animals become infected through the spread of parasites due to inadequate care, unpleasant environment, harsh weather and close contact with unsanitary animals [4]. Despite pathogenic effects *Oestrus ovis* (*O. ovis*) responsible for serious economic losses in livestock as consequence of its impact loss of body weight, reduced meat, wool and dairy production [5]. When migratory larvae approach the cerebral cavity they can potentially pierce and destroy the frontal sinuses and dorsal turbinate bones and rarely skull bones resulting in 'false gid' [6]. During their development and migration, larvae cause mechanical damage and irritation to the host nasal sinuses. The damaging effect on nasal sinuses lead to immediate clinical issues such as incoordination, dyspnea (shortness of breath), restlessness, and conjunctivitis which occur during development, emaciation, sneezing and mucopurulent nasal discharge [7]. *O. ovis* is important for both medicine and public health, can cause ocular-myiasis in farmers and shepherds when humans are incidental host [8].

In humans, *O. ovis* may cause ophthalmomyiasis [9]. In recent decades, a number of human cases involving tourists travelling to or returning from endemic regions have been documented [10, 11]. *O. ovis* exhibit adaptability to different environments [12], tolerating a broad range of relative humidity levels, with a predilection high temperatures and solar irradiance [13]. *O. ovis* is a worldwide problem several studies on caprine oestrosis have been reported from Mediterranean or Middle Eastern countries such as France (28.5%) [14], Spain (43%) [15], Italy (55.8% in Massine, Sicily) [16], Jordan (58%) [17], and in Konya region 59% and in Nigde province of Turkey (22.52%) [18, 19]. Knowledge about the prevalence and epidemiology of caprine oestrosis is quite limited in Pakistan. Pakistan is a subtropical country where a high prevalence of goat population is infected by it, very scant literature is available consisting of only a few reports of external ophthalmomyiasis cases in Pakistan specifically in the Sindh, Karachi as Ali *et al.*, (2006), reported first case of external ophthalmomyiasis by [20], subsequent cases have been documented in the region, including a report by Abbas and Amla (2016), and Faish *et al.*, 2014 [21, 22], outside of Karachi, Hanif *et al.*, (2012), reported a case in Jammu and Kashmir [23].

However, only one article has reported the prevalence and larval burden of *O. ovis* in goats of Karachi, Sindh, Pakistan [4]. To the best of our knowledge, no previous studies on the prevalence and intensity of the disease in the southern Punjab particularly in Lodhran district has reported. Lodhran is characterized by dry and hot climate in summer and cold in winter. During the summer, temperature fluctuates from 28°C to 42°C. Wintertime temperatures range from 5°C to 21°C [24]. The aim of this study was to

determine first time (1) Overall monthly prevalence, (2) To assess seasonal variation, (3) Age-group larval burden in male and female goats, (4) Gender wise parasite burdens of *O. ovis* infection in goats, (5) Monthly distribution of larval stages of *O. ovis*, (6) To explore potential factors associated with *O. ovis* infestation including gender and age.

Materials and method

2.1, Study Period and sample collection

The research was carried out in Lodhran, a district of Punjab. The prevalence of *O. ovis* infestation was investigated on a monthly basis in goats of Lodhran district of Punjab from October 2021-April 2022. The climate of this area is predominantly hot and dry with extended summers and mild winters with irregular rainfall, concentrated in spring and autumn. The goats slaughtered here come from surrounding villages over a radius of 150 km. Visits to abattoirs were made on every working day. Goats of all age groups and both sexes were sampled

systematically. A total of 47 goat heads were examined for *O. ovis* larvae from abattoirs in Lodhran, Punjab, Pakistan. The sample size of 47 was constrained due to resources limitations including equipment, personnel and funding as well as the availability of slaughtered animals during the study period. Each month, almost 8 goat skulls were examined as part of the study. Data, including the month, animal's sex, and age, were recorded during sample collection. The age of animal was estimated based on the stage of dentition, season and by questioning from owners. Goats of all ages and breeds were included in this study and the goats with visible sign of abnormalities or diseases at the time of slaughter were excluded from the study. The heads of slaughtered animals were separated from rest of body and transported to the laboratory in plastic bags for a complete examination [25]. Ethical approval was not required for this study as samples were collected from slaughtered animals at abattoirs.

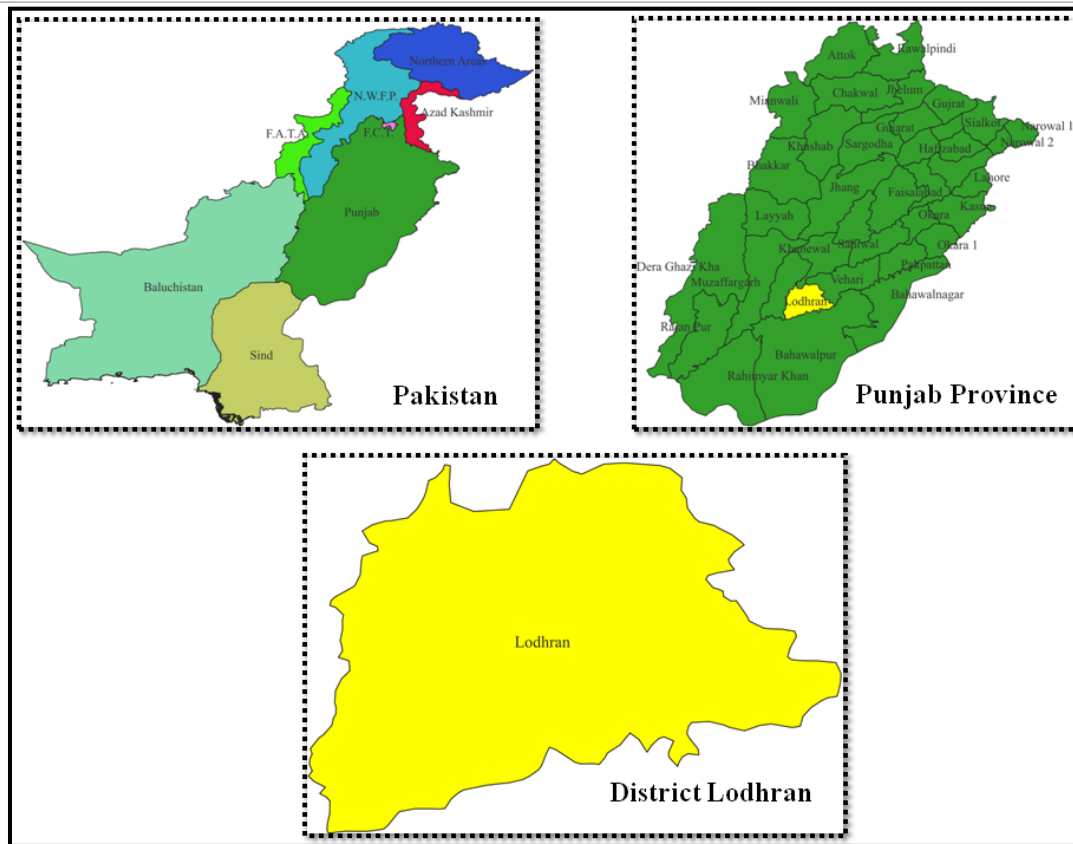


Figure 1. Map of Pakistan with highlighted sampling site of district Lodhran, Punjab, Pakistan

Collection and identification of larvae of *O. ovis*

In the laboratory heads were sectioned from dorsal surface the skull was cut in two symmetrical halves along longitudinal plane using electrical saw. The presence of *O. ovis* larvae in nasal cavities, septum, middle meatus, chonchae and sinuses was extensively studied in dissected head samples. The first, second and third instar larvae (L1, L2 and L3) were found alive inside the heads larvae were collected with forceps, rinsed with physiological saline solution, and fixed in 70% of ethanol in 2-3ml ethanol-filled glass vials as shown in figure 2. The larval stages were then numbered and identified using important morphological traits under a stereomicroscope [26]. The percentage of each larval instar was calculated using the equation (Larval stage percent = number of each larval stage/Total number of larvae×100). Identification of the

specimens was performed in each positive head by using keys of Zumpt (1965) [26]. The data were entered into a Microsoft Excel spreadsheet. The prevalence was calculated as the number of animals found infested with *O. ovis* larvae divided by the total number of animals examined.

Statistical Analysis

Data were analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Prevalence was calculated as the proportion of infected animals to total examined and expressed as percentages with 95% confidence intervals. Chi-square (χ^2) test was used to compare prevalence between age groups and seasons. Fisher's exact test was applied for sex comparison due to small expected cell frequencies (<5). Statistical significance was set at $P < 0.05$.



Figure 2. Preservation of *O. ovis* in 70% ethyl alcohol

3. Results

3.1 Prevalence in Lodhran

Out of 47 heads of goats examined, 19 (40%) were found positive for *O. ovis*. The samples were considered positive even if a single larva was detected. The prevalence rate observed during different months of the year did not vary much,

Table 1. Monthly prevalence of *O. ovis* in goats by sex

Months	X: Number of examined goats	No of male	No of female	X: No of infested animal	X/N: no of infested animal/no of examined animal
Oct.2021	5	2	3	2	2/5 (40%)
Nov.2021	7	1	6	5	5/7 (71.4%)
Dec.2021	6	1	5	3	3/6 (50%)
Jan.2022	7	2	5	3	3/7 (42.9%)
Feb.2022	6	0	6	2	2/6 (33.3%)
Mar.2022	8	3	5	2	2/8 (25%)
Apr.2022	8	3	5	2	2/8 (25%)
Total	47	12	35	19	19/47 (40.4%)

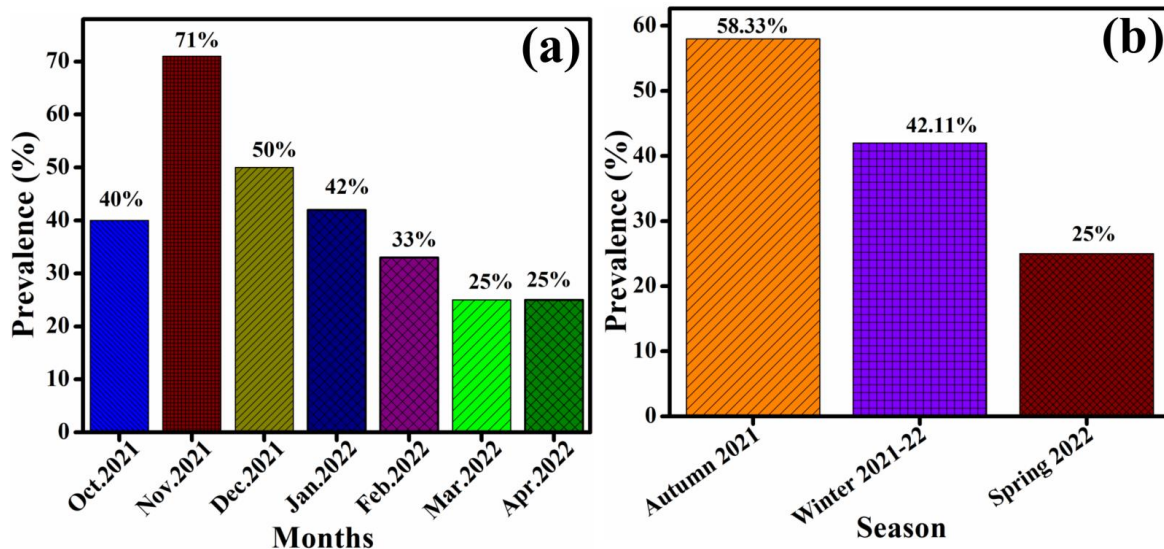
The seasonal prevalence of *O. ovis* in Lodhran district from October 2021 to April 2022 shows a decline from autumn through spring, with the highest prevalence in autumn (58.3%) and the

except in the month of November 2021. The highest prevalence was observed in November 2021 (71%), while lowest prevalence was observed (25%) in March and April 2022 as it shown in table 1.

lowest in spring (25.0%) as depicted in Table 2. Chi-square test X^2 showed there is no statistically significant difference between seasons ($P = 0.202 > 0.05$).

Table 2. Seasonal prevalence of *O. ovis*

Season	Positive/Total	Prevalence (%)	Statistics
Autumn 2021	7/12	58.3%	Chi-square test $X^2 = 3.20$ df = 2 P = 0.202
Winter 2021-22	8/19	42.1%	
Spring 2022	4/16	25.0%	


Figure 3: Monthly (a) and seasonal (b) prevalence of *O. ovis* in goats of Lodhran district, Punjab, Pakistan, between October 2021 to April 2022

Further, out of total 47 head samples, 12 belonged to male goats and 35 belonged to female goats. Out of 12 males 4 were infected and out of 35 females 15 were infected. Additionally, the incidence of infestation was higher in female compared to males in the collected samples. The overall prevalence in female was (42.9%), while that in males was

(33.3%) as represented in Table 3. Fisher's exact test showed no statistically significant difference between sexes ($P = 0.736$). The infected males had 23 larvae accounting for (43%) of the total larvae in males while in females, the larval burden was 30, accounting for (56%) of the total larvae in females.

Table 3. Larvae counts and infestation percentage according to sex of goats in Lodhran

Sex	X: Examined goats	N: Infested animal	X/N: infested animal/examined animals	Larvae count (%)
Male	12	4	4/12 (33.3%)	23/53 (43.3%)
Female	35	15	15/35 (42.9%)	30/53 (56.6%)
Total	47	19	19/47 (40.42%)	53/53 (100%)
Statistics	Fisher's exact test $P = 0.736$ OR = 0.67 95% CI: 0.17-2.63			

The study also determined the prevalence of *O. ovis* infestation in goats of three different age groups as shown in table 4. Among the three age

groups examined, animals below one year of age, 18% of the animals were positive for infestation and animals aged 1-2 years, 52% of the animals

were positive for infestation, whereas 38% of the animals beyond 2 years of age were positive for infestation. Chi-square test X^2 showed no

statistically significant difference between different age groups ($P = 0.165 > 0.05$).

Table 4: Age wise prevalence of *O. ovis* in goats of Lodhran

Age	0-1 years		1-2 years		> 2years	
Month	Examined	Positive	Examined	Positive	Examined	Positive
Oct.2021	1	0	2	1	2	1
Nov.2021	1	0	5	4	1	1
Dec.2021	1	0	3	2	2	1
Jan.2022	2	1	4	2	1	0
Feb.2022	1	0	3	1	2	1
Mar.2022	3	1	3	1	2	0
Apr.2022	2	0	3	1	3	1
Prevalence	11	2(18.1%)	23	12(52.17%)	13	5(38.4%)
Statistics	Chi-square test $X^2 = 3.60$ d f = 2 P = 0.165					

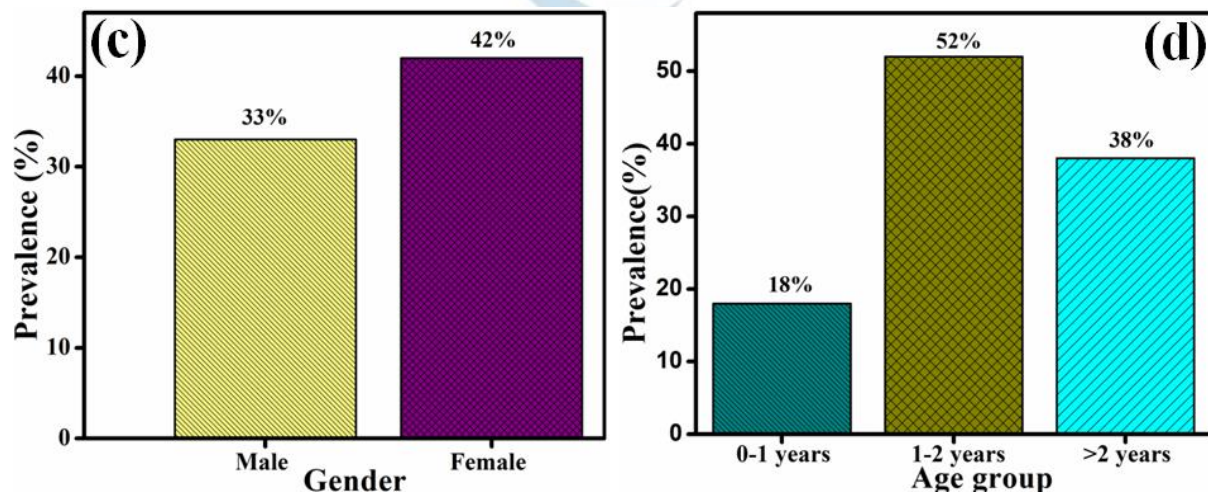


Figure 4: Gender (c) and age group (b) prevalence of *O. ovis* in goats of Lodhran district, Punjab, Pakistan

A total of 53 *O. ovis* larvae were recovered from goat heads as shown in table 5. The larval burden per goat ranged from 1 to 8. The highest larval burden was observed in December. The lowest larval burden was 2, observed October. The bulk of larvae found during the investigation were L3, while L2 were least in number and no L1 was found. The larvae

detected during the study were L2 and L3 instars with majority of L3 and L2 instars while no L1 was observed. A total of 47 L3 larvae were collected but only 6, L2 larvae were observed while the presence of L1 was not observed. The overall prevalence of L3 larvae was 88% while that of L2 larvae was 11%. The mean larval burden per infected goats were: 2.79 ± 2.1

(range: 1-8), as it is shown in table 5.

Table 5: Monthly distribution of larval stages of *O. ovis*

Months	L1	L2	L3	Total number of larvae	Mean larvae burden per infected goat
Oct.2021	0	0	2	2	
Nov.2021	0	5	7	12	
Dec.2021	0	0	15	15	
Jan.2022	0	1	10	11	
Feb.2022	0	0	4	4	2.79 ± 2.1 (range: 1-8)
Mar.2022	0	0	5	5	
Apr.2022	0	0	4	4	
Total	0	6/53 (11.3%)	47/53(88.6%)	53	

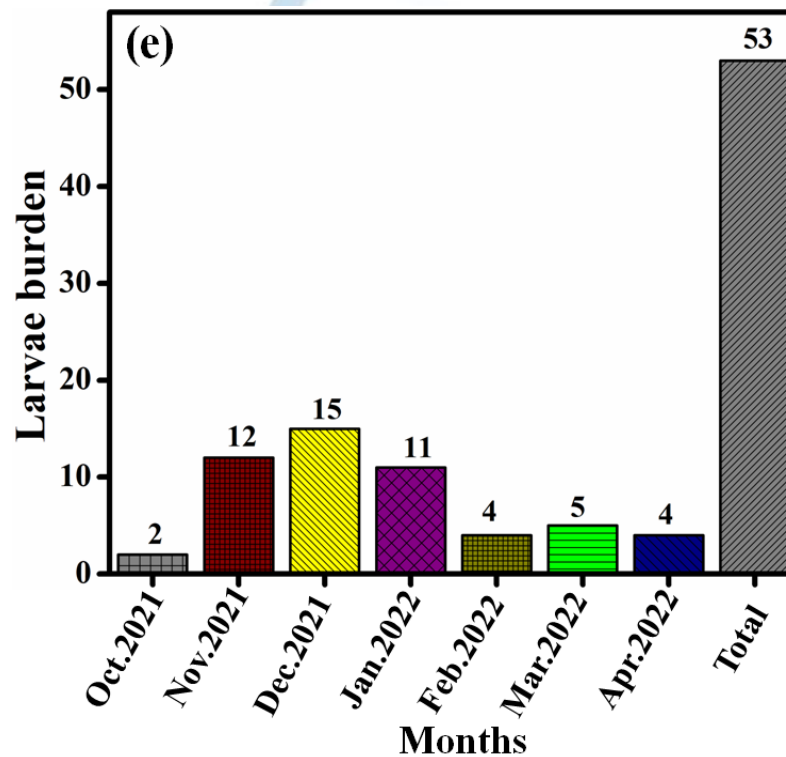


Figure 5: Monthly larvae burden of *O. ovis* in goats from district Lodhran, Punjab, Pakistan
Microscopic examination of *O. ovis*

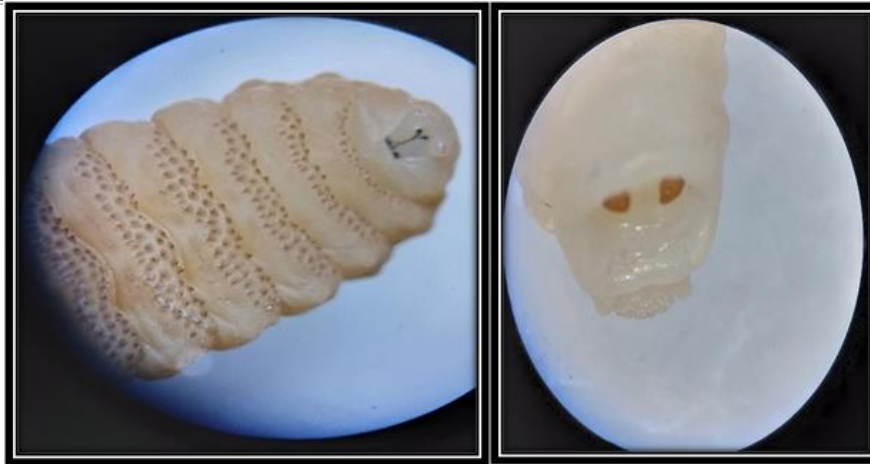


Figure 6: Oral hooks of *O. ovis* and D-shaped spiracles are observed under stereomicroscope



Figure 7: Left side represent the ventral side of *O. ovis* and right side represent the D-shaped spiracles of L2 Larval stages 4.

Discussion

The purpose of present study was to provide base-line data regarding caprine oestrosis in Lodhran district, Punjab, Pakistan. Caprine oestrosis is an endemic parasitic infection which has significant implications for both public health and the economy. This study conducted on goats showed that the overall prevalence recorded was 40% out of 47 samples. Our results are almost similar to the study conducted in Turkey (38.5%) in Diyarbakir Province [2], and in Saudi Arabia, in Riyadh (44.5%) region [27]. In Pakistan, a similar prevalence rate of 36.24% was reported in goats of Karachi by only one author [4]. When the results of our study were compared with those of other countries,

the incidence of infection was higher than in Iran (13.06%) [28], and northern Jordan (24%) [29]. But incidence of disease was less than European countries i.e. Spain (71.1%) [30], and African territories e.g. Ethiopia (88.4%) [31]. This variation may be due to various factors including an increase in fly burden, absence of treatments against this parasite, differences in geographical area, animal breed, due to favorable climatic factors in these regions like loose sandy type of soil which is favorable for pupation and emergence of adult flies, poor animal hygiene, inadequate veterinary services, and variations in animal management and husbandry systems may contribute to the differences in prevalence rates among studies.

The results of seasonal impact on goats differed according to characteristics studied. The monthly infestation rate varied in goats from 25% to 71%. The highest prevalence was observed in November (71%), during the autumn season and the lowest in March and April (25%) during spring season as explained in (Table I). This agrees with a previous study [32], which reported that peak infestation in Iraq occurred in autumn, which was 76.92%, while lowest infestation rates were in winter (30.76%). In contrast to above reports, a study in Turkey showed the highest prevalence of *O. ovis* in winter, and the lowest in autumn and summer [33]. Another study conducted, in Riyadh Region of Saudi Arabia reported that peak infestation occurred in spring [27]. The reason for these differences may be changes in weather and environmental conditions as well as ability of *O. ovis* maggots to adjust their biological cycles during the developmental phase according to particular climatic condition of each area. The absence of fly activity for most of the year is probably due to hot, dry, and windy weather. The optimum temperature for adults emerging from pupa is 27°C, while constant temperatures below 16°C and above 32°C were fatal [34]. *O. ovis* is active in subtropical regions during summer and spring seasons. It can infect livestock throughout the year in hot climatic conditions [35]. Then, females deposited larvae in/around nostrils of goat. The current study shows that in Lodhran district during the hottest months of the year (June and July), flies are either absent or negligible while, eclosion of adult flies occurred primarily in August and September, providing an increased opportunity of flock infestation.

Overall prevalence recorded in Lodhran showed a higher infestation rate in female goats (42.9%) compared to male goats (33.3%), as represented in (Table 3) although this difference was not statistically significant (Fisher's exact test, $P = 0.736$, OR = 0.67, 95% CI: 0.17-2.63). This lack

of statistical significance may be attributed to the small sample size. Females had a higher frequency of *O. ovis* larvae than males in flocks which might be due to the higher density of females in flocks or due to physiological variations between males and females or a specific behavior of female animals which facilitates their infestation by *O. ovis* larvae [36]. In previous study, similar results of high female prevalence were also reported in Nigeria [37] and north-eastern Turkey [38]. Conversely, effect of sex on prevalence of Oestrosis was not significant in one study [1], and a higher prevalence in males was observed in another study in Libya [9]. High prevalence in males may be due to body conformation (more surface area), higher male density and age of male animals [25].

Higher prevalence was observed in goat's aged in between 1-2 years (52%) compared to young animals (Table 4). The lower infestation rate in young animals may probably be due to fact that younger animals are active enough to avoid larval deposits by adult female flies, during periods of intense fly activity, they may be observed hiding their noses in their dam's wool another possible reasons is that the surface area of nasal orifice in adult animals is much broader than of young animals. Furthermore, adult animal's respiration are slower than those of young animals which may facilitate female fly to ovipositor and larvae move into nasal sinuses also, a young animal may have maternally derived antibodies against caprine oestrosis obtained from its mother [39]. On contrary to above reports, a study in Central Oromia showed the highest prevalence in young animals compared to adults [31], the higher prevalence in young than adult animals is probably due to the fact that young animals have low immunological response to parasites, which may increase with age and due to the fact that higher numbers of young animals were examined than adults.

The present study recorded an overall prevalence of larvae in Lodhran with 0% L1,

11% L2 and 88% L3 out of total 53 larvae recovered from goats. The highest numbers of third instar larvae were observed in December (15) and the lowest number of larvae was observed in October (2) as shown in (Table 5). In contrast to previous studies L1 result was decreased than that recorded by Arslan *et al.*, (2009), who reported that L1 larvae prevailed at 90.5% of the total larvae [38], and another study (Mohammed *et al.*, 2020) reported 13.14% L1 larvae [32]. The differences might have originated from seasonal, climatic and breed differences in the study areas. Our results agreed with a study that reported in Riyadh region of Saudi Arabia, which reported a higher proportion of L3 larvae (98.8%) compared to L2 (1.2%) and L1 (0%) [27]. It is possible that L1 larvae might have been passed unseen due to their small size and presence in hidden places, such as the turbinate and ethmoid bones, or that numerous L1 larvae were demolished in the nasal holes during hypo-biotic period [40].

This study has several limitations. The sample size (n=47) was relatively small, which may limit generalizability. The study was conducted in a single district and may not represent broader patterns across Punjab. The 7-month study period did not capture a full annual cycle. Future studies with larger sample sizes across multiple districts and complete annual cycles are recommended. These findings have practical implications for livestock farmers and veterinary services in the region. To estimate the economic importance of oestrosis and the efficiency of control measures in this area it is recommended to include more animals determining more potential factors and monitoring *O. ovis* fly activities over the year, as well as the use of an antiparasitic drug effective against *O. ovis* in the routine treatment of goat to reduce the incidence of this disease.

5. Conclusion This study reports the first systematic investigation of caprine oestrosis in Lodhran district, Punjab, Pakistan and it is

anticipated to effect on animal welfare and output. It is concluded that the *O. ovis* infections in goats pose a risk to goat productivity in the area under study. We examined how potential factors associated with infestation like gender and age impacted the results. The overall prevalence of 40.4% indicates that *O. ovis* is endemic in the region. Key findings include: (1) highest prevalence during November (autumn onset), (2) higher prevalence in female goats and animals aged 1-2 years, were more susceptible and (3) predominance of L3 larval stage. These findings suggested that control programs should target [specific timing and groups]. Implementation of anthelmintic like ivermectin and closantel based treatments during orally every 3 months four times per annum and improved animal husbandry practices may reduce the disease burden.

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