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Seroprevalence of *Oestrus ovis* infection in sheep in Southwest of Iran

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Abstract

***Oestrus ovis*, sheep nasal bot fly, is one of the important parasites and causes of sinus and nasal myiasis in small ruminants. It is a zoonotic infection that causes disturbing myiasis in humans. This study was aimed to determine the seroprevalence of *O.ovis* infection in sheep population in southwest of Iran. During 2009-2010, blood samples were collected monthly and randomly from 1800 sheep (>6 months of age) of Ilam and slaughtered sheep of Ahvaz (the capital of Khuzestan). All sera were examined for antibodies to crude somatic antigens of *O.ovis* second-stage larvae using an Elisa test. Positive sera were prepared by marking the sheep, blood taking and direct observation of the parasite in the head. The somatic antigens were prepared by ultra-sonication of the second-stage larvae. Results of the ELISA test on the sheep sera revealed that seroprevalence of *Oestrus ovis* infection in sheep of Ilam and of Khuzestan was 427 (47.4%), out of 900 and 398 (44.2%) out of 900 respectively. Mean seroprevalence of *Oestrus ovis* infection in sheep in southwest of Iran was 825 of 1800(45.8%). Seasonal differences in the prevalence of the infection were significant ($p<0.001$), with the highest rates in spring (Apr.-May. 62.7%) and autumn (Oct. 61.5%, Nov. 76.0%), the lowest rates in summer (Aug. 27.1%-29.3%) and winter (Jan. 41.1%, Feb. 26.7%). The results of this study demonstrate that *O.ovis* has a widespread distribution in the sheep population of southwest of Iran. Control measures must be applied continuously to decrease the incidence of the infection in livestock herds.**

Keywords: *Oestrus ovis*, sheep, seroprevalence, ELISA, Iran.

Introduction

Oestrus ovis (Linne 1761, Diptera: Oestridae), the sheep nasal bot fly, is one of the important obligate parasites that causes sinus and nasal myiasis in domestic and wild small ruminants. The larvae which develop in the sites may cause severe discomfort, partly because of mechanical damage caused by hooks and

spines of the larvae, and also as a result of & Shearer, 2001). Economic losses of the parasite are very important due to both damages of larval stages and great stress of larvipositional activities of female flies which significantly interferes with grazing and rumination thus reducing productivity (wool, meat and milk) of infected animals (Kaufmann, 1996; Shearer, 2001). Most studies on *Oestrus ovis* infection in small ruminants have been based on examination of slaughtered animal heads (Arslan, et al. 2009; Jafari Shoorijeh, *et al*. 2009; Tavassoli, *et al*. 2012). Several studies on immune responses of animal hosts against *O. ovis* have been reported (Angulo-Valadez, *et al*. 2008; Angulo-Valadez, *et al*. 2009; Dorchies 1996; Duranton, *et al*. 1999; Suarez, *et al*. 2005). Specific Immunoglobulin G (IgG) levels were found to increase with developing second stage (L2) and/or third stage (L3) larvae of *O.ovis* in sheep and goats (Romero, *et al*. 2010). Circulating IgG antibody responses to the larvae can be detected in both ovine and caprine hosts (Terefe, *et al*. 2005). Serodiagnostic detection of *Oestrus ovis* in sheep and goats has been conducted using larval antigens of the parasite (Alcaide, et al. 2005; Bauer, *et al*. 2002; Papadopoulos, *et al*. 2001). In Iran, the prevalence of the parasite infection has only been investigated by examination of slaughtered animal heads. The *Oestrus ovis* prevalence in sheep has been reported 49.7% in Shiraz (Jafari Shoorijeh, *et al*. 2009), 30.34% in Urmia (Tavassoli *et al*. 2012;), southern and northwest of Iran respectively (fig. 1).

Oestrus ovis is also a zoonosis that produces annoying myiasis in humans by the first stage larvae of the parasite. Many human cases of nasal, pharyngeal and ophthalmomyiasis caused by *O. ovis* have been reported from Iran and other countries (Ali, *et al*. 2006; Alizadeh, *et al*. 2014; Arslan, *et al*. 2010; Hazratian, *et al*. 2017; Jenzeri, *et al*. 2009; Smillie, *et al*. 2010; Sucilathangam, *et al*. 2013).

Our previous study, by ELISA test and second stage larval antigens especially crude extract (somatic) antigens of *O. ovis*, showed high sensitivity and specificity for serological detection of IgG in infected sheep in Iran (Yoosef Vand, 2011). Despite the high number of sheep present (up to 4 million) and the importance of sheep breeding in the area, there was no reported data on the prevalence of the infection of *O. ovis* in the animals. Therefore, the present study aimed to investigate the seroprevalence of

O. ovis infection in sheep selected from southwest of Iran.

Material and methods

Study area and samples

The study was conducted during the year 2009-2010, in Southwest-Iran. Blood samples were collected monthly and randomly from 1800 sheep (>6 months of age, males and females), from slaughterhouses of two geographical localities, Ilam (Ilam province) and Ahvaz (Khuzestan province) (Fig.1).

Ilam province is one of the warmest regions of Iran. The mountainous areas of north and northeastern Ilam are relatively cold. The average annual rainfall of the province is 578 mm. Over a year, the temperature typically varies from -2°C to 37°C and is rarely below -5°C or above 39°C. Ilam central coordination locates in 33.6384°N 46. 4226°E. Ahvaz city, the capital of Khuzestan province is located at 20 m above the sea level, in the southwest of Iran (32°20' N, 40°20' E) with subtropical climate condition, a moderate winter, and hot summer, with temperatures regularly at least 45°C, sometimes exceeding 50°C. The average annual rainfall is around 230 mm.

Fig. 1. Map of Iran showing the region of the study (Ilam and Khuzestan provinces; black area).

Breeds of sheep were mostly Sanjabi in Ilam and Arabi in the Khuzestan provinces. Arabi and Sanjabi sheep are the most important dual-purpose (meat and wool) native sheep breeds in the west and Southwest regions of Iran. Sera were prepared from blood and stored at −20 ◦C until use.

All collected sera (900 samples of each province, monthly 72 samples) were examined for antibodies to crude extract antigen (CE) of *O. ovis* second-stage larvae using an enzyme-linked immunosorbent assay (ELISA). Positive control sera from the infected animals were prepared as follows: Blood samples were taken from the labeled horned sheep of slaughterhouse. The marked heads of slaughtered sheep were separated from the body and horns were cut. Moreover, to find the parasite larvae, the head (nose and

horn area) of each animal was hit hard on the ground. Any animal in which the parasite larvae were isolated from its head was considered infected. The isolated larvae were identified based on the keys described by Zumpt (1965).

Enzyme-linked immunosorbent assay (ELISA)

Collected *O. ovis* second-stage larvae were washed several times in phosphate-buffered saline (PBS) supplemented with penicillin (100 U/ml) and streptomycin (100 mcg /ml). The viability of larvae was checked under a stereomicroscope (Olympus SZH10, Japan). The crude extract antigens were prepared as follows: The larvae (L2) were cut by scalpel then homogenized by an ultrasonic homogenizer (Bandelin- HD 2070, Germany) in PBS pH 7.2 (10 L2/5ml PBS), centrifuged at 2000 g for 20 min at 4 ^{O}C, after that, the supernatants were removed and filtered through 0.22 mm filters (Biofil siring filter). The protein concentration of the extracts was determined by a modified Bradford method and finally the extract was stored at −70 ◦C.

The best antigen, serum, and conjugate dilutions in the ELISA test were determined by the checkered board titration method. Microplate wells (Nunc, Denmark) were coated with the antigens (100 µl per well/ at 6 µg protein), and incubated for overnight in 4^{0}C. Sera were tested in triplicate at a dilution of 1:10 in bicarbonate/carbonate coating buffer pH 9.6. Peroxidase-conjugated anti-sheep immunoglobulin G (Sigma–Aldrich, Germany) was used at a dilution of 1:8000. Plates were incubated for 3 h at 37 ^{o}C. After washing in PBS, 5% skimmed milk solution in PBS was added for blocking the wells. After triple washings, 100 ml of duplicated sera diluted at 1: 10 in PBS with 0.1% Tween 20 was added and incubated at 37 C for 45min. After washing, 100 mL of anti-sheep immunoglobulin G (IgG, whole molecule) peroxidase conjugate (Sigma; A-3415) diluted at 1: 8000 was added per well. Then, 100 mL of substrate solution with citrate buffer 0.05M (pH=5.0), hydrogen peroxide and 3, 30, 5, 50-tetramethylbenzidine (TMB) (Sigma; T-3405, Germany) was incubated for 20 min and stopped with 3N sulphuric acid (Merk, Germany). Positive controls were evaluated by repeated titration of several serum samples of naturally *O. ovis* infested animals from the studied regions (as described above). Negative controls were obtained from serum samples of

lambs kept indoors that had no contact with adult botflies. Optical density (OD) values were spectrophotometrically (MRX II; Dynex Technologies, West Sussex, U.K.) measured at 450 nm. Afterwards, the cut-off values were determined as a value of two standard deviations (P<0.05) above the mean OD from negative control serum samples.

Data analysis

Statistical analysis was performed by SPSS software version 16.0 (Statistical Package for Social Sciences, Chicago, IL, USA). For this, data were analyzed by the Chi-square test and Logistic regression.

Results

All collected sera were examined for antibodies to crude extract antigen of *O. ovis* L2 larvae using an Elisa test. Results of Elisa test on the sheep sera revealed that seroprevalence of *O. ovis* infection in sheep of Ilam (n:900) and Khuzestan (n: 900) was 427 out of 900 (47.4%, 95% CI: 44.1-50.7%), 398 out of 900 (44.2%, 95% CI: 41-47.4%), respectively. The mean seroprevalence of *O. ovis* infection in sheep in southwest of Iran was 825 out of 1800 (45.8%, 95% CI: 43.5-48.1%). Furthermore, the statistical analysis showed that the season was significantly associated with infection and the highest infection rate was in spring and autumn while the lowest infection rate was in winter and summer (p<0.001). Seasonal prevalence of *O.ovis* infection in sheep of Ilam was obtained 45.5% in spring (max. in May. 62.7%), 39% in summer (min. in Aug. 29.3%), 60.8% in autumn (max. in Nov. 76.0%) and 45.5% in winter (min. in Feb. 28.6%); as a result, the observed differences were statistically significant (p<0.001) (Table 1, Fig.2). Seasonal prevalence of *O. ovis* infection in sheep in Ahvaz was obtained 52.4% in spring (max. in Apr. 62.7%), 41.2% in summer (min. in Aug. 27.1%), 39.6% in autumn (max. in Oct. 61.5%) and 36.9% in winter (min. in Feb. 26.7%), which the observed differences were also statistically significant (p<0.001) (Table. 2, Fig. 2). In addition, the relative frequency of infection was higher in Sanjabi (47.4%, 95% CI: 44.2-50.6%) than Arabi sheep breeds (42.2%, 95% CI: 41-47.4%). More specifically, the odds ratio of the infection in Sanjabi sheep was 1.14 greater than Arabi sheep (95% CI: 0.95-1.37), although this difference was not statistically significant (P>0.05). The odds ratio of the infection in summer, autumn and winter was 0.46 (95% CI: 0.35-0.61), 1.35 (95% CI: 1.04-1.76) and 0.45 (95% CI: 0.34-0.58) greater than spring, respectively. This difference was statistically significant (K2=63.03, df=3,

p< 0.01) and 7.3% of fluctuation in infection was then justified by the season. One percent (0.1%) of the fluctuation of the infection was justified by breed or geographical location. The chances of the Arabi and Sanjabi sheep breeds were 42.2% (95% CI: 41-47.4%) and 47.4% (95% CI: 44.2-50.6%) positive, respectively but the differences were not statistically significant.

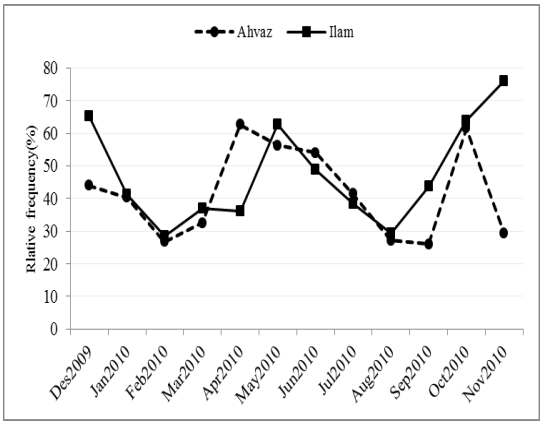

Figure 2. Monthly frequency of *O. ovis* infection in sheep of Southwest of Iran by ELISA

Table.1. Results of ELISA test with crude extract antigen of *O.ovis* second stage larvae in sheep from Ilam (Ilam province).

Months of year	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.
Total samples examined	75	75	70	73	72	75	80	73	75	80	72	75
NO. Positive (%)	49 (65.3)	31 (41.3)	20 (28.6)	27 (37.0)	26 (36.1)	47 (62.7)	39 (48.8)	28 (38.4)	22 (29.3)	35 (43.8)	46 (63.9)	57 (76.0)
NO. Negative (%)	26 (34.7)	44 (58.7)	50 (71.4)	46 (63.0)	46 (63.9)	26 (37.3)	41 (51.2)	45 (61.6)	53 (70.7)	45 (56.2)	26 (36.1)	18 (24.0)

Table.2. Results of ELISA test with crude extract antigen of *O. ovis* second stage larvae in sheep from Ahvaz (Khuzestan province).

Months of year	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.
Total samples examined	75	67	75	80	75	80	74	82	70	69	78	75
NO. Positive (%)	33 (44.0)	27 (40.3)	20 (26.7)	26 (32.5)	47 (62.7)	45 (56.3)	40 (54.1)	34 (41.5)	19 (27.1)	18 (26.1)	48 (61.5)	22 (29.3)
NO. Negative (%)	42 (56.0)	40 (59.7)	55 (73.3)	54 (67.5)	28 (37.3)	35 (43.7)	34 (45.9)	48 (58.5)	51 (72.9)	51 (73.9)	30 (38.5)	53 (70.7)

Discussion

The domestic sheep are considered to be the primary host of *O. ovis* (as called a nasal bot, or head maggot of sheep) but other ruminants and humans are also infected (Marquardt *et al*. 2000). Many cases of disturbing infection of the parasite in eyes, nose, ears, and pharynx of human have been reported from Iran (Hakimi & Yazsi, 2002; Masoodi & Hosseini, 2003; Alizadeh, et al. 2014; Hazratian, et al. 2017;). This study was based on appropriate results of our previous two studies on *O. ovis* in the region. First, Alborzi, *et al*. (2014) showed the presence of several antigenic proteins in the crude extract (CE) of O. *ovis* larvae (L2) using sheep sera and immunoblotting method. Second, Yoosef Vand, (2011) in a house- ELISA with CE of O. *ovis* larvae (L2) showed that the test can be used to detect the infection in sheep with relatively good sensitivity (90.1%), and specificity (89.1%).

This large- scale (n: 1800) seroepidemiologic study was conducted for the first time owing to the lack of information on *O. ovis* infection prevalence in sheep of the area. The total seroprevalence rate of the infection in sheep of the area was 45.8%. The results of our study demonstrated that *O. ovis* is a common parasite in sheep of Southwest of Iran indicating the presence of the parasite infection throughout the entire year. Also, seasonal differences in the prevalence of the parasite infection were observed. The highest and lowest infection rate was found in spring-autumn, and winter-summer, respectively. The seasonal increase in the seroprevalence of the infection may be related to the increased activity of the larval stages, especially the second stage larvae of the parasite, reactivation of arrested larvae (Yilma and Dorchies, 1991), or larvipositional activity of *O. ovis* female fly and deposition of its first-stage larvae in the nostrils of sheep. In contrast, the seasonal decrease in seroprevalence of the infection may be associated with reduced larval activity and entry into the third stage larvae of the parasite.Seasonal differences in the prevalence of the infection in sheep from Turkey have been reported by Arslan *et al*. (2009). The similarity in the prevalence of *O. ovis*. sheep infection in the two regions, our study area, southwest (45.8%) and Shiraz, south (49.7%) of Iran (Jafari Shoorijeh *et al.* 2009), can be due to their almost similar climatic conditions, which is suitable for better growth of the parasite. Despite the high population of small ruminants in Iran and the economic importance of the parasite, information on

the prevalence of its infection is limited to the following two slaughterhouse studies. Jafari Shoorijeh *et al.* (2009) and Tavassoli *et al*. (2012) reported the prevalence of *O.ovis* infection in sheep from Shiraz, in the south of Iran (49.7%) and from Urmia, in northwest of Iran (30.34%), respectively.

The results of our study showed that the higher prevalence of infection than in Urmia, in the northwest of Iran (30.34%) is possibly due to different geographical conditions, particularly, the cold weather of Urmia that may be extended for several months causing hypobiosis of the larvae.

Moreover, several seroprevalence studies on *O. ovis* infection in sheep have been reported in some other countries. Higher prevalence was found in two breed sheep (Djallonke and Sahelian Bali Bali) from West Africa, Burkina Faso (n: 421, 86.3%; Ouattara *et al*. 1996) that used the ELISA technique and crude antigen from stage 2 larvae of *O. ovis* (Ouattara & Dorchies, 1996). Seroprevalence of *O. ovis* infection was also found in sheep from southwestern Spain (n: 5,878, 69.30%; Alcaide *et al*. 2005) and from southwestern Germany (n: 1497, 50.3%; Bauer *et al*. 2002). In these studies, sera from sheep were analyzed for detecting *O. ovis* antibodies with ELISA tests using a crude antigen from stage 2 (L2) larvae of *O. ovis*. Some studies have indicated an association between prevalence and other factors such as flock size (Bauer *et al*. 2002), latitude (Alcaide et al. 2005) and animal breed (Arslan *et al*. 2009). Arslan, *et al*. (2009) found that *O.ovis* infection rate in the morkaraman breed with the dark-colored head was significantly higher compared to the rate in the Akkaraman breed with light-colored head. But, the result of the present study revealed that the infection prevalence in sheep is not dependent on the different breeds of the animals.

Considering that Arabi sheep breed is characterized as white (usually), cream, black and dark/bright brown color, while Sanjabi sheep are a naturally white and chalky color breed with brown to light brown faces (Roshanfekr *et al*. 2014; Shokrollahi & Baneh, 2012). It seems that these differences are more relevant to the type of studies (serological and in slaughter survey) than animal breed or color. In this regard, Ouattara, *et al*. (1996), showed that *O. ovis* infection in sheep had no significant relationship with their breeds.

In conclusion, the results of our study clearly demonstrate that *O. ovis* has

a widespread distribution in the sheep population of southwest of Iran. Despite the high prevalence of the infection, no marked clinical cases were seen in sampled animals. Finally, because of the economic effects of this infection in the animals, control measures must be applied continuously to decrease the incidence of the infection in livestock herds.

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References

Alborzi, AR., Jolodar, A., Seyfi Abad Shapouri, M. and Bagherian pour E. (2014). Isolation and identification of excretory-secretory and somatic antigens from the *Oestrus ovis* larvae by SDS-PAGE and immunoblotting. Vet. Res. F. 5, 4, 307 – 311.

Alcaide, M., Reina, D., Frontera, E. and Navarrete, I. (2005). Analysis of larval antigens of *Oestrus ovis* for the diagnosis of oestrosis by enzyme-linked immunosorbent assay. Med. Vet. Entomol. 19,2, 151-157.

Alcaide, M., Reina, D., Sanchez-Lopez, J., Frontera, E. and Navarrete, I. (2005). Seroprevalence of *Oestrus ovis* (Diptera, Oestridae) infestation and associated risk factors in ovine livestock from southwestern Spain. J Med Entomol. 42, 327-331.

Ali, A., Feroze, AH., Ferrar, P., Abbas, A. and Beg, MA. (2006). First report of ophthalmomyaisis externa in Pakistan. J. Pak. Med. Assoc. 56, 86–7.

Alizadeh, M., Mowlavi, G., Kargar, F., Nateghpour, M., Akbarzadeh, K., & Hajenorouzali-Tehrani, M. (2014). A review of myiasis in Iran and a new nosocomial case from Tehran, Iran. J Arthropod Borne Dis. 8, 124.

Angulo-Valadez, C E., Cepeda-Palacios, R., Ascencio, F., Jacquiet, Ph., Dorchies, Ph., Ramirez-Orduna, J M. and Lopez M A. (2009). IgG antibody response against salivary gland antigens from *Oestrus ovis* (Diptera: Oestridae) in experimentally and naturally infected goats. Vet. Parasitol. 16, 356–359.

Angulo-Valadez, CE., Scala, A., Grisez, C., Prevot, F., Bergeaud, JP., Carta, A., Cepeda-Palacios, R., Ascencio, F., Terefe, G., Dorchies, P. and Jacquiet, P. (2008). Specific IgG antibody responses in *Oestrus ovis* L. (Diptera: Oestridae) infected sheep: associations with intensity of infection and larval development. Vet. Parasitol. 155, 3-4, 257-263.

Arslan F., Mete, B., Oztürk, R. and Samasti M.(2010). External ophthalmomyiasis caused by *Oestrus ovis* in Istanbul. Trop. Doct. 40,3,186-7.

Arslan, MO., Kara, M. and Gicik Y. (2009). Epidemiology of *Oestrus ovis* infestations in sheep in Kars province of north-eastern Turkey. Trop. Anim. Health. Prod. 41,3, 299-305.

Bauer, C., Steng, G., Prevot, F. (2002). Dorchies P. Seroprevalence of *Oestrus ovis* infestation in sheep in southwestern Germany. Vet. Parasitol. 110, 137–143.

Dorchies, P. and Yilma, JM. (1996). Current knowledge in immunology of *Oestrus ovis* infection. Acta. Parasitol. Acta. Turcica. 20, 563–580.

Duranton,C., Dorchies, P., Grand, S., Lesure, C. (1999). Oswald IP. Changing reactivity of caprine and ovine mononuclear phagocytes throughout part of the life cycle of *Oestrus ovis*: assessment through spontaneous and inductible NO production. Vet. Res. 30, 371–376.

Hakimi, R. and Yazsi I. (2002). Oral mucosa myiasis caused by *Oestrus ovis*. Arch. Iran. Med. 5,3, 194-196.

Hazratian, T., Tagizadeh, A., Chaichi, M. and Abbasi, M. (2017). Pharyngeal myiasis caused by sheep botfly, *Oestrus ovis* (Diptera: Oestridae) larva, Tabriz, East Azarbaijan Province, Iran: a case report. J Arthropod Borne Dis. 11, 166.

Jafari Shoorijeh, S., Tamadon, A., Negahban, SH. and Behzadi, MA. (2009) Prevalence and intensity of *Oestrus ovis* in sheep of Shiraz, southern Iran. Trop. Anim. Health. Prod. 41,7, 1259-1262.

Jenzerim S., Ammari, W., Attia, S., Zaouali, S., Babba, H., Messaoud, R. and Khairallah, M. (2009). External ophthalmomyiasis manifesting with keratouveitis. Int. Ophthalmol. 29, 533-535.

Kaufmann, J. (1996). Parasitic Infections of Domestic Animals: A Diagnostic Manual, Birkhäuser, Berlin, Germany. PP.183-84.

Marquardt, WC., Demaree, RS., Grieve, RB. (2000). Parasitology and Vector Biology. Harcoart Academic Press, London. 2nd Edition, PP: 273-279.

Masoodi, M. and Hosseini, K. (2003). The respiratory and allergic manifestation of human myiasis caused by larvae of the sheep bot fly (*Oestrus ovis*): A report of 33 pharyngeal cases from

southern Iran. Ann. Trop. Med. Parasitol. 97,1, 75-81.

Melhorn, H. (2008). Encyclopedic Reference of Parasitology, Springer-Verlag Heidelberg. 2nd ed. PP: 720-22.

Ouattara, L., and P. Dorchies. (1996). Serological prevalence of *Oestrus ovis* infestation in Burkina Faso: evaluation using the ELISA technique. Rev. Elev. Med. Vet. Pays.Trop. 49, 219-221.

Papadopoulos, E., Prevot, F., Jacquite, P., Duranton, C., Bergeaud, JP., Kalaitzakis, E. and Dorchies, P. (2001). Seasonal of variation of *Oestrus ovis*- specific antibodies in sheep and goat mixed floks in Greece, Vet Parasitol. 95,1, 73-77.

Romero, JA., Arias, MS., Suarez, JL., Paz-Silva, A., Francisco, I., Alonso, F., Cortinas, FJ., Dacal, V., Romasanta, A., Morrondo, P., Diez-Banos, P., Scala, A. and Sanchez-Andrade, R. (2010). Application of the analysis of serum antibodies (IgM and IgG) to estimate the seroprevalence of ovine oestrosis and to evaluate the effect of chemotherapy. J. Med. Entomol. 47, 477–481.

Roshanfekr, H. (2014). Estimation of genetic parameters for Kleiber ratio and trends for weight at birth and weaning in Arabi sheep. Int. J. Adv. Biol. Biom. Res. 2,11, 2830-2836.

Suarez, JL., Scala, A., Romero, JA., Paz-Silva, A., Pedreira, J., Arias, M., Diaz, P., Morrondo, P., Diez-Banos, P. and Sanchez-Andrade, R. (2005). Analysis of the humoral immune response to *Oestrus ovis* in ovine. Vet. Parasitol. 134,153–158.

Tavassoli, M., Tajic, H., Malekifard, F., Solimanizadeh, A. and Mardani K. (2012). seasonal infestation of *Oestrus ovis* larvae in slaughtered sheep in urmia, Iran. IVJ. 7,4, 78- 98. (Persian full text).

Terefe, G., Yacob, HT., Grisez, C., Prevot, F., Dumas, E., Bergeaud, JP., Dorchies, P., Hoste, H. and Jacquiet P. (2005). *Haemonchus contortus* egg excretion and female length reduction in sheep previously infected with *Oestrus ovis* (Diptera: Oestridae) larvae. Vet. Parasitol. 128,3-4, 271-283.

Shokrollahi, B. and Baneh, H. (2012). Co-variance components and genetic parameters for growth traits in Arabi sheep using different animal models. G.M. R. 11, 305- 314.

Smillie, I., Gubbi, P. K. S., & Cocks, H. C. (2010). Nasal and

ophthalmomyiasis: case report. J Laryngol Otol. 124, 934-935.

Sucilathangam, G., Meenakshisundaram, A., Hariramasubramanian, S., Anandhi, D., Palaniappan, N., & Anna, T. (2013). External ophthalmomyiasis which was caused by sheep botfly (*Oestrus ovis*) larva: a report of 10 cases. J Clin Diagn Res. 7(3), 539.

Wall, R. and Shearer, D. (2001). Veterinary Ectoparasites: Biology, pathology and control. New Jersey, USA: Wiley Blackwell. 2nd ed. PP.121-122.

Yilma, JM., Dorchies, Ph. (1991). Epidemiology of *Oestrus ovis* in southwest France. Vet. Parasitol. 40, 315-323.

Yoosef Vand, Z. (2011). Evaluation of an in-house enzyme linked immuonosorbent assay (ELISA) for detection of anti-*Oestrus ovis* antibodies in sheep. Msc Thesis, Shahid Chamran University of Ahvaz, Iran.

Zumpt, F. (1965). Myiasis in man and animals in the old world; A textbook for physicians, veterinarians and zoologists. London, UK: Butterworths.PP: 267-270.