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Clinical, Histopathological and Parasitological Evaluation of *Oestrus ovis* Infecting Mountain Goats and Sheep

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ABSTRACT

In our study, we aimed to present the cases of *Oestrus ovis* in domesticated and wild ruminants. The material of this study consisted of 10 sheep and six mountain goats admitted to our department, between 2013 and 2019, for necropsy. The animals presented various neurological symptoms, including rotation around their axis, opistotonus, loss of coordination, rhinitis, loss of appetite, head pressed against objects, unilateral blindness and torticollis. Subsequently, routine systemic necropsy was performed. After routine tissue procedures, 5 µm thick sections were taken from paraffin blocks prepared for hematoxylin eosin staining. Sections were examined under light microscope to determine the histopathologic changes. In four of the six goats, in addition to, *Oestrus ovis* larvae, *Coenurus cerebralis* cysts were detected in cerebrum and cerebellum. *Coenurus cerebralis* cysts were also observed in four of the ten sheep.

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INTRODUCTION

Oestrus ovis L. (Diptera: Oestridae) is a cosmopolitan parasite and causes cavitary myiasis. The larvae are obligate parasites of the paranasal sinuses and nasal cavities of sheep, goats and some wild animals (Carvalvo et al. 2015; Mumcuoglu and Eliashar, 2011). Larvae in the nasal cavities and sinuses may migrate to the brain, and cause false Gid (Negm-Eldin et al., 2015). Sneezing, breathing difficulty, nasal discharges, emaciation and incoordination are the most important clinical symptoms observed in infested animals (Amaravathi et al., 2016; Özdal et al., 2016). Significant economic losses due to *Oestrus ovis* have been reported, including 22% of body weight, 16% of wool production and 10% of milk production (Sayin Ipek, 2018). This disease has a worldwide distribution, but is more prevalent in the Mediterranean regions of Africa and Europe (Özdal et al., 2016).
In this study, we aimed to present the cases of *Oestrus ovis* in sheep and wild mountain goats admitted to the Department of Pathology of the Faculty of Veterinary Medicine in Kafkas University between 2013 and 2019 for necropsy and parasitological evaluation.

MATERIALS AND METHODS

Ethical Approval

Permission was obtained from the Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK 2020/063), General Directorate of Nature Protection and National Parks of the Ministry of Agriculture and Forestry for this study (21264211-288.04-E.1229382).

Animals

The material of this study consisted of 10 sheep and six mountain goats admitted to the Department of Pathology of the Faculty of Veterinary Medicine in Kafkas University, between 2013 and 2019, for necropsy. The animals presented various neurological symptoms, including rotation around their axis, opistotonus, loss of coordination, rhinitis, pneumonia, loss of appetite, head pressed against objects, dullness, unable to stand up, balance loss, unilateral blindness and torticollis (Figure 1). Subsequently, routine systemic necropsy was performed.

Figure 1. Clinical view of the animal

Parasitological Examinations

Oestrus ovis larvae were collected with the help of pliers, placed in 70% ethyl alcohol, and brought to the Entomology Laboratory of the Parasitology Department of the Faculty of Veterinary Medicine in Kafkas University. The larvae were then placed in petri dishes and fixed with hot 70% ethyl alcohol. The fixed larvae were rendered transparent in 30% KOH solution, after being punctured at several points with a pin. The transparent larvae were dissected under a stereo microscope and their morphological features were examined under a light microscope. The stages and morphological criteria of the larvae were determined in the light of the literature (Uslu and Dik, 2006; Zumpt, 1965).

Histopathological Examinations

Tissue samples taken after systemic necropsy of the mountain goats and sheep were fixed in 10% buffered formaldehyde solution. Following the routine tissue procedures, 5µm thick sections were obtained from paraffin blocks for hematoxylin eosin (H&E) staining. Sections were examined under a light microscope (Olympus Bx53) and photographed using the Cell^P program (Olympus Soft Imaging Solutions GmbH, 3,4) to evaluate histopathological lesions.

RESULTS

Parasitological Results

When the skulls of the 10 sheep and six mountain goats were opened, fly larvae were encountered (Figure 2 A-B). Fly larvae were found upon transversal separation of the skulls. It was determined that 14 of the 20 fly larvae collected were second stage larvae, while the remaining six were third stage larvae (Figure 3A). The average size of the larvae was 6x1.65 mm in the first period, 7.8x3.2 mm in the second period and 12.7x5.3 mm in the third period. The macroscopic and microscopic appearance of the cephalopharyngeal skeleton and posterior stigma according to the stages of *Oestrus ovis* larvae are shown in Figure 3 B-H.

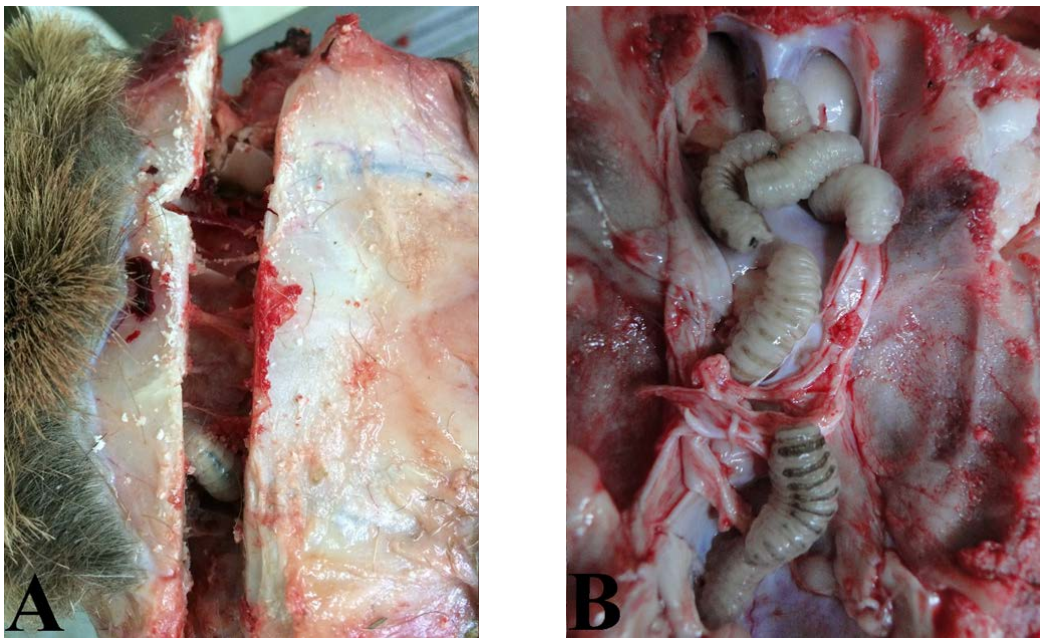

Figure 2. A) Mountain goat, *Oestrus ovis* larvae in the frontal sinus. **B)** Sheep, *Oestrus ovis* larvae.

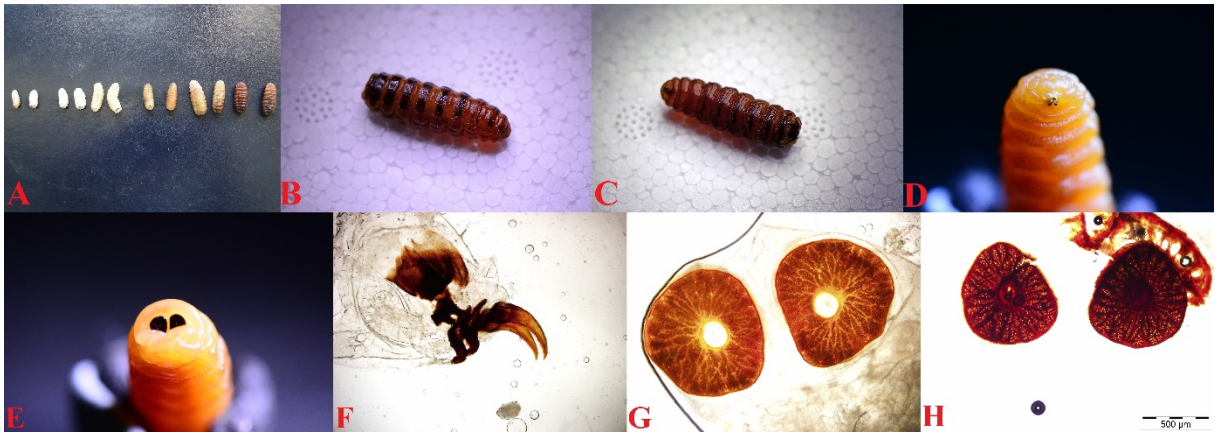

Figure 3. A) Macroscopic appearance of larvae taken after systemic necropsies according to the stage of development **B)** Dorsal view of *O. ovis* 3rd stage larva. **C)** Ventral view of *O. ovis* 3rd stage larva **D)** Macroscopic view of the Cephalopharyngeal skeleton of *O. ovis* 3rd stage **E)** Macroscopic view of the posterior stigma of the 3rd stage larva of the *O. ovis* **F)** Microscopic view of the Cephalopharyngeal skeleton of *O. ovis* 2nd stage larva **G)** Microscopic view of posterior stigmas of 2nd stage larvae of *O. ovis* **H)** Microscopic view of the posterior stigma of the 3rd stage larva of the *O. ovis*

Macroscopic Results

In four of the six goats, in addition to *Oestrus ovis* larvae, *Coenurus cerebralis* cysts were detected in the cerebrum and cerebellum (Figure 4A). *Coenurus cerebralis* cysts were also observed in four of the ten sheep (Figure 4B). In addition cerebral melanosis (Figure 4C), hydatid cysts, verminous and interstitial pneumonia, hemorrhage in the liver, enteritis and sinusitis were also observed.

Figure 4. A) Mountain goats, *Coenurus cerebralis* cysts in cerebrum and cerebellum **B)** Sheep, *C. cerebralis* cysts in cerebrum **C)** Sheep, cerebral melanosis.

Microscopic Results

In the histopathological examination of the *Oestrus ovis* cases, hydatid cysts were detected in lung and liver. In addition, interstitial pneumonia characterized by peribronchiolar cell infiltration, hyperemia and thickening of the interalveolar septum was observed in the majority of animals. In some animals, verminous pneumonia was detected. Pinworms were found in the bronchial, bronchiolar and alveolar lumens. Sarcocysts were recorded in heart tissue in a very small number of animals. Severe melanosis in brain tissue was observed in only 2 animals (Figure 5A-F). *Oestrus ovis* with *Coenurus cerebralis* cases, nonpurulent meningoencephalitis was found. In addition, liquefaction necrosis, a large number of foreign body giant cells and perivascular cell infiltration were observed.

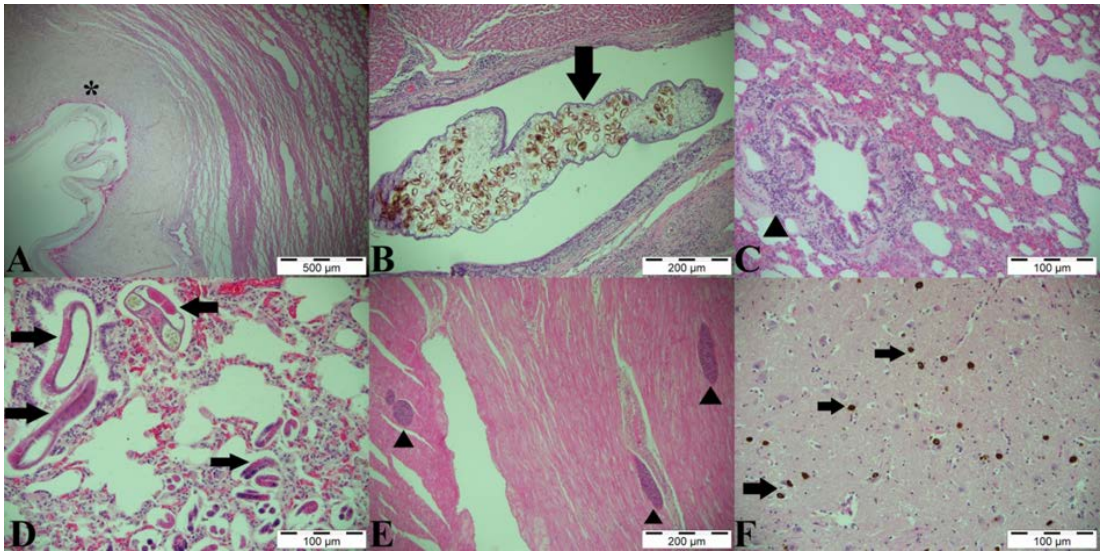

Figure 5. A) Lung, hydatid cyst (star), H&E, 500 µm **B)** Liver, hydatid cyst (arrow), H&E, 200 µm **C)** Lung, Interstitial pneumonia, peribronchiolar cell infiltration (arrowhead), H&E, 100 µm **D)** Verminous pneumonia, pinworms in the bronchioles and alveolar lumens (arrows), H&E, 100 µm **E)** Heart, Sarcocysts (arrows), H&E, 200 µm **F)** Brain, cerebral melanosis, H&E, 100 µm.

DISCUSSION AND CONCLUSION

Oestrus ovis is an obligate parasite of the nasal and frontal sinus cavities of sheep and goats. Although the parasite is seen especially in summer, spring and autumn, it can be active even in winter (Saleem et al., 2017). In our study, 11 of the 16 *Oestrus ovis* cases were brought in spring, 3 in summer and 2 in winter. The larvae of the sheep nasal bot fly, *Oestrus ovis*, causes a myiasis, known as oestrosis, in small ruminants (Allaie et al., 2016; Papadopoulos and Dvořák, 2015). *Oestrus ovis* rarely migrates to the brain tissue through the ethmoidal bone (Gül et al., 2007). Oestrosis is a zoonotic disease and results in ophthalmomyasis (Sayın İpek and Altan S, 2017). Although this disease is quite common in sheep, it is rather rarer in goats (Giri et al., 2016). When sheep and goats are compared, sheep has 2 times more infestation than goats (Deniz and Yukarı 2013). In our study, four cases of *Oestrus ovis* were detected in frontal sinuses in mountain goats. Parallel to the literature (Giri et al., 2016), *Oestrus ovis* was found to be more common in sheep in our study.

Female *O. ovis* accumulates its first stage larvae (L1) directly into the nostrils of sheep and goats. The L1 actively penetrate through the nostrils and colonize the cornet and septum where they develop (Hanan 2013). First stage larvae are dorso-ventral flattened. Their length varies between 1.41-3.38 mm (Aldemir 2015, Deniz and Yukarı 2013). Once settled at the ethmoid level, the L1 molt to the larvae (L2) of stage two. The second stage larvae are white and 3.5-10 mm (Aldemir 2015, Deniz and Yukarı 2013). The L2 also rise from the nasal cavity to the frontal sinuses, where they molt to stage three larvae (L3). The third stage larvae are 11-15 mm in length and creamy in young periods, they turn darker as they mature (Aldemir 2015, Deniz and Yukarı 2013). The L3 exit the nasal cavity of the host in the nasal mucus that is expelled by sneezing and that then drops to the soil. Subsequently, the L3 pupate in 12 to 24 hours. Finally, when external conditions are appropriate, the pupae are transformed into adult flies within 30 to 34 days (Basmaciyan et al., 2018). Consistent with the literature (Aldemir 2015; Basmaciyan et al., 2018; Hanan 2013), we determined that 14 of the 20 fly larvae were in second stage, and six were third stage larvae. We also found that average size of the larvae was 6x1.65 mm in the first period, 7.8x3.2 mm in the second period, and 12.7x5.3 mm in the third period.

The clinical symptoms observed in animals with *O. ovis* are briefly as follows; runny nose, rubbing, sneeze, weakness, rotation around its axis and uncoordinated. Consistent with literature data (Yıldız 2016), the animals that formed the material of our study presented various neurological symptoms, including rotation around their axis, opistotonus, loss of coordination, rhinitis, pneumonia, loss of appetite, head pressed against objects, dullness, unable to stand up, balance loss, unilateral blindness and torticollis. In addition to mechanical damage to tissues, interstitial pneumonia may develop in the lung (Yıldız 2016). In our study, we encountered interstitial pneumonia in the lung in most of the animals. In addition, animals with *O. ovis* are more susceptible to secondary bacterial infections (Yıldız 2016). Parallel with literature data (Yıldız 2016), *E.coli* infection was detected in three animals. *Listeria monocytogenes* infection was recorded in only one animal.

There have been no studies investigating the prevalence of *O. ovis* in goats in our country. However, the prevalence of *O. ovis* in sheep has been found to range from 22.52-59%, in studies carried out in various regions (Sayin Ipek, 2018).

In conclusion, *O. ovis* larvae were found 16 cases between 2013 and 2019. In the literature, although there are numerous studies of *O. ovis* in which domestic ruminants were evaluated, there are few studies in which domesticated and wild ruminants are evaluated together. Especially in mountain goats, in addition to *O. ovis*, lung pinworms and hydatid cysts were observed in animals. We interpreted that the presence of such severe parasitism in mountain goats is normal because the animals do not live under control and do not receive regular antiparasitic treatment. We thought that the results obtained from the study will be useful for the province of Kars, which is an important wildlife area and will contribute to the literature data.

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