

The first report of massive infestation with *Lipoptena Cervi* (Diptera: Hippoboscidae) in Roe Deer (*Capreolus Capreolus*) in Iasi county, N-E of Romania

[O primeiro relatório de infestação maciça com *Lipoptena Cervi* (Diptera: Hippoboscidae) em Roe Deer (*Capreolus capreolus*) no condado de Iasi, NE da Romênia]

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ABSTRACT

Investigations of four roe deer corpses were carried out from May until October 2014, in the Veterinary Forensic Laboratory and in the Parasitic Diseases Clinic, in the Iasi Faculty of Veterinary Medicine. The roe deer were harvested by shooting during the trophy hunting season. The clinical examination of the shot specimens revealed the presence of a highly consistent number of extremely mobile apterous insects, spread on the face, head, neck, lateral body parts, abdominal regions, inguinal, perianal and, finally, all over the body. The corpses presented weakening, anemia and cutaneous modification conditions. Several dozen insects were prelevated in a glass recipient and preserved in 70° alcoholic solution in order to identify the ectoparasite species. The morphological characteristics included insects in the Diptera order, Hippoboscidae family, *Lipoptena cervi* species. These are highly hematophagous insects that by severe weakening are affecting the game health and trophy quality. Histological investigations of the skin revealed some inflammatory reactions caused by ectoparasite *Lipoptena cervi*. *Lipoptena cervi* was identified for the first time in Iasi County, Romania.

Keywords: roe deer, *Lipoptena cervi*, SEM, histopathology, Iasi county

RESUMO

As investigações de quatro cadáveres de veados foram realizadas desde maio até outubro de 2014, no Laboratório Forense de Veterinária e na Clínica de Doenças Parasitárias, da Faculdade de Medicina Veterinária de Iasi. Os veados foram abatidos por tiro durante a temporada de caça troféu. O exame clínico dos espécimes revelou a presença de um número muito consistente de insetos ápteros extremamente móveis, espalhados pela face, pela cabeça, pelo pescoço, pelas partes do corpo laterais, pelas regiões abdominal, inguinal, perianal e, finalmente, por todo o corpo. Os cadáveres apresentavam condições de enfraquecimento, anemia e alterações cutâneas. Várias dezenas de insetos foram separados em um recipiente de vidro e preservados em solução alcoólica de 70°, a fim de se identificarem as espécies de ectoparasitos. As características morfológicas incluem os insetos da ordem Diptera, família Hippoboscidae, espécie *Lipoptena Cervi*. Estes são insetos hematófagos que estão enfraquecendo e afetando a qualidade de saúde de animais de caça e troféu. Investigações histológicas da pele revelaram algumas reações inflamatórias causadas pelo ectoparasita *cervi* *Lipoptena*. *Lipoptena cervi* foi identificado pela primeira vez no condado de Iasi, na Romênia.

Palavras-chave: veado, *Lipoptena cervi*, SEM, histopatologia, condado de Iasi

INTRODUCTION

A common hematophagous deer kid is *Lipoptena cervi* (Diptera: Hippoboscidae; syn. deer ked, deer fly, forest fly) parasitizing the red deer

(*Cervus elaphus*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and other wild ruminants in Europe, Siberia, northern China and North America, as well as some domestic animals: horses and cattle (Paakkonen *et al*, 2012, Hodžić *et al*, 2012). With recent

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climate change, understanding the ecology of ectoparasites has become topical, as their niches will change geographically and some are vectors of disease. During the autumn swarming period, the deer keds may attack a variety of animals and humans, but only cervids seem to be able to function as definitive hosts (Madslie et al, 2011).

The forestry workers, hunters and other people who work in or visit forests are especially vulnerable to incidental deer ked infestation (Härkönen, 2009). Ectoparasite *Lipoptena cervi* can cause damage, hyperemia and inflammation reactions to its host skin, and also severe blood loss (Dehio, 2004; Hodžic, 2012).

Bartonella spp. is a Gram-negative bacteria. *Bartonella* species is an intracellular parasite and can infect healthy people, but are considered especially important as opportunistic pathogens, which are transmitted by hematophagous parasites like fleas, mosquitoes, ticks, and various flies (Halos, 2004).

The aim of this study was to determine the species of ectoparasites found on four male roe deer (*Capreolus capreolus*) shot during the hunting season 2014 in the area of Iasi county, Romania.

MATERIALS AND METHODS

Four roe deer corpses originated from the cynegetic patrimony of Turia-Perieni, Ciurea forestry, Iasi, Romania. The roe deer were harvested by shooting during the trophy hunting season (May - October 2014).

The epidemiological, parasitological, and laboratory data were analysed. The skin and organ samples were fixed in 10% formalin, then were embedded in paraffin and cut at 5 µm thickness and stained with Haematoxylin, Eosin and Blue methyl (HEA) and Periodic Acid Schiff (PAS). The examination and microfilming of the histological slides were conducted by using binocular magnifying glass and photon microscope Leica ICC 50 HD, ocular 10, lenses x4, x10, x20.

Scanning electron microscopy (SEM) investigations: the investigated material consists of deer ked. The material was fixed in

glutaraldehyde (2%) for 2 hours, osmium tetroxide (1%) for 4 hours, and washed with phosphate buffer. After dehydration in a graded ethanol series (40%, 70%, 80%, 90% and 100%) and acetone, the material was critical-point dried with CO₂ (using an EMS 850 Critical Point Dryer), sputter-coated with a thin layer of gold (30 nm) (using an EMS 550X Sputter Coater) and, finally, examined by scanning electron microscopy (Tescan Vega II SBH) at an acceleration voltage of 30.00 kV.

RESULTS AND DISCUSSION

Romania is located in the southeast of Central Europe, in the inferior area of the Danube, to the north of the Balkan Peninsula and on the north-west shore of the Black Sea. Its neighbours are: Bulgaria to the south, Serbia to the southeast, Hungary to the northwest, Ukraine to the northeast, Moldavian Republic to the east, and the Black Sea shore to the southeast. Vegetation is distributed on three levels: alpine area, forest area and steppe area. The climate is temperate-continental and the average annual temperature in the south of the country is 11°C; in the north it is 8°C; in east it is 9°C, and in the west it is 10°C.

Iasi lies in the eastern part of Moldova, Moldavian Plain. The town lies on the Bahlui river, a tributary of the Jijia river, which flows into the river Prut. It presents a pronounced continental climate, being influenced by air masses with Eastern origin; cold winters and hot summers.

The maximum average temperatures recorded in the studied periods were framed around 27.2°C and recorded an average rainfall of 37.8 l/m². (National Fund's data of National Meteorological Administration).

The clinical examination of the deer specimens shot on Turia Perieni hunting fund from Iași county (Figure 1) revealed the presence of a highly consistent number of extremely mobile apterous insects, spread on the face, head, neck, lateral body parts, abdominal regions, inguinal, perianal and, finally, all over the body. The average number of the deer ked on the host was over 2500 parasites per host (Figure 2a, b).

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Figure 1. The map for the cynegetic area Turia-Perieni, Curea. Iasi County.

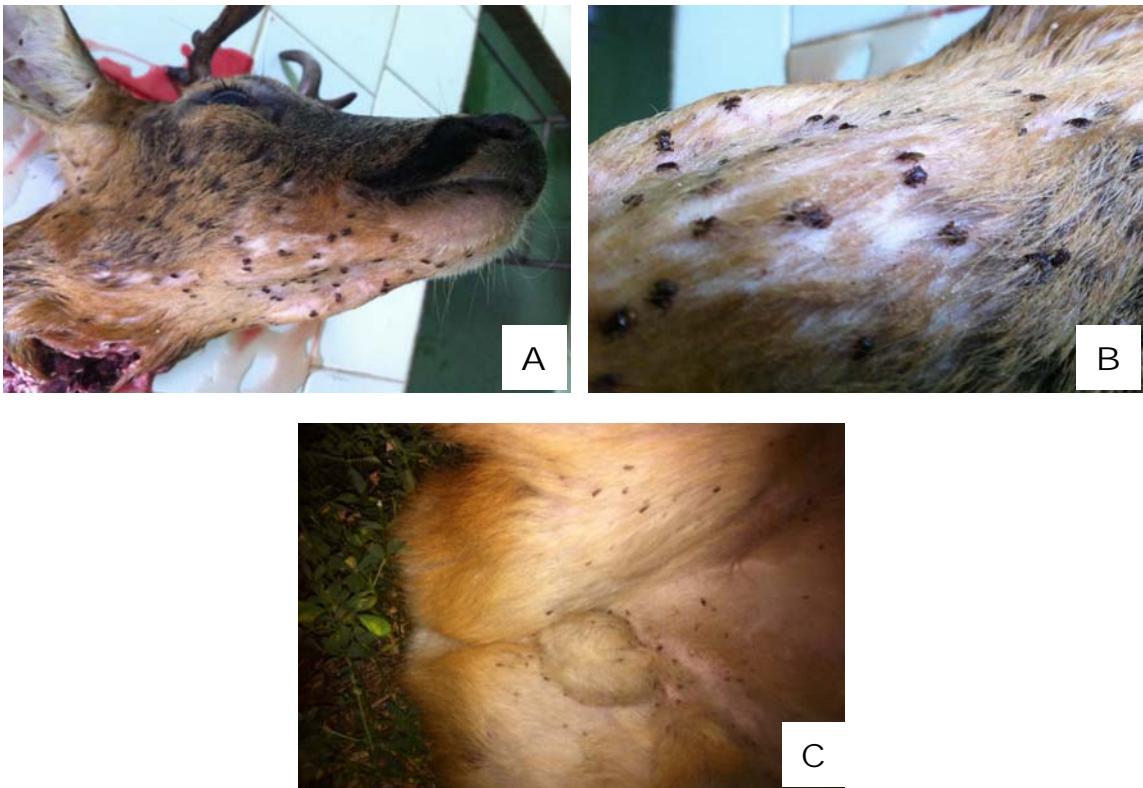


Figure 2. Red deer - macroscopic aspects: A) Head – the presence of hematophagous insects; B) Head – areas of alopecia; C) Parasitic invasion in the inguinal region.

The corpses presented a weakened status, anemia, and cutaneous changes. In order to identify the ectoparasite species, several dozen insects were prelevated in a glass recipient and preserved in 70° alcoholic solution.

Morphological characteristics places the insects in the *Diptera* order, in the family *Hippoboscidae*, species *Lipoptena cervi*, highly hematophagous insects, that by severe weakening are affecting the game health and trophy quality.

On examined specimens lesions were identified on skin level translated through dermatitis, depilation and diffuse alopecia, punctate

hemorrhages at the feeding place of parasites (Fig. 2c).

Adult parasites are 3.5-5 mm in length and have a brownish color. The head, thorax and abdomen are flattened and strong in appearance (Fig. 3a, b). The head and thorax are brown and the eyes are multifaceted (Fig. 3c). The parasites' legs are robust with large dark claws. In general, it is covered with strong black bristles (Fig. 3d). The number of the deer ked on the host (*Capreolus capreolus*) was a mild infestation comparing with the data published in Finland where the invasion with deer ked was abundant (Kynkaanniemi et al., 2010).

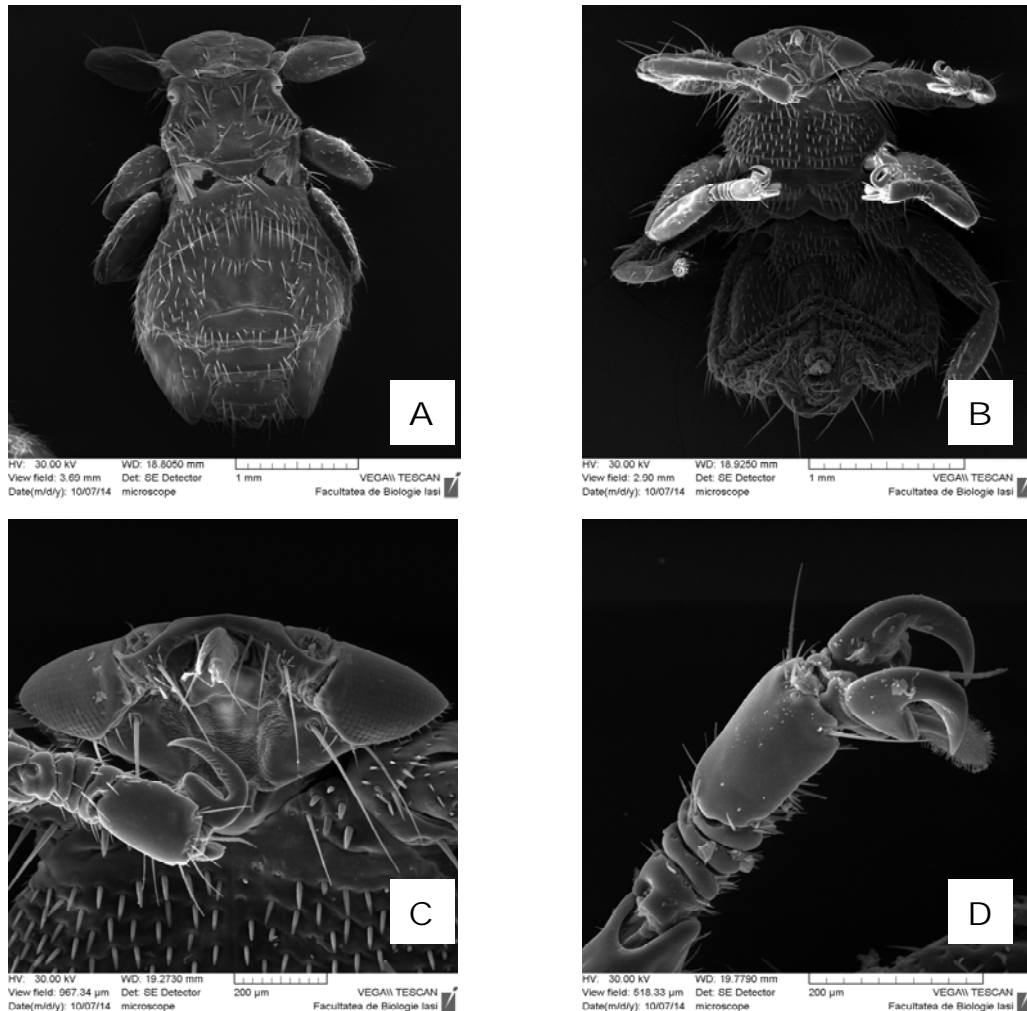


Figure 3. *Lipoptena cervi* Linnaeus, 1711. SEM. A) Dorsal view and B) Ventral view. Hair threads cover the hole body, 1mm; C) Buccal apparatus and multifaceted eyes, 200 µm; D) robust legs with big claws and hair threads, 200 µm.

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On examined deer histopathological examinations were performed in the most affected areas of the skin. Thus, orthokeratotic hyperkeratosis with eosinophilic, lymphocytic and mast cells infiltration were identified in the form of agglomerations in skin dermis, dermal edema with dissociation of platysma muscle fibers (Fig. 4a). Mononuclear infiltrate in the dermis, grouped in the form of nodules and presence of mild edema of dermal connective tissue with collagen fibers dissociation. Stratum corneum consistent in progressive exfoliation and degeneration of sebaceous glands (Fig. 4b).

We also noticed perifollicular edema (around the hair follicle), but also in dermal structures that led to dissociation of muscle and conjunctive fibers and a moderate disorganization of the hypodermis and dermis architecture. Inflammatory infiltrate composed mostly of eosinophils is disposed predominantly as perivascular and vessel walls presenting an endothelial hypertrophy (Fig. 4 c, d).

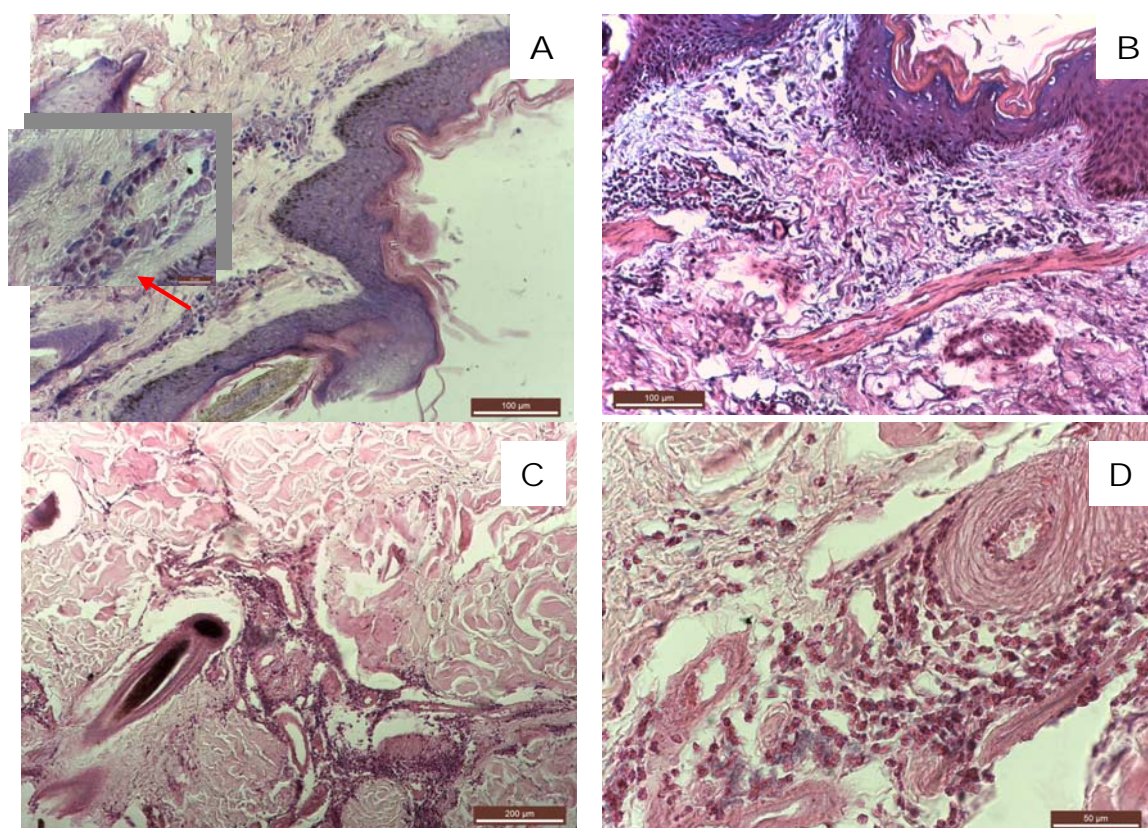


Figure 4. Skin histology of red deer. **A)** Orthokeratotic, eosinophilic, mast cells and lymphocytic infiltration of the dermis. PAS, 100µm; **B)** Moderate hyperkeratosis, degeneration of sebaceous glands and dermal edema. HEA, 100µm; **C)** Perifollicular, hypodermal and dermal edema. HE, 200µm; **D)** Perivascular eosinophilic inflammation. HE, 50µm.

CONCLUSIONS

The infestation with *Lipoptena cervi* of the roe deer (*Capreolus capreolus*) in the cynegetic area of Turia Perieni-Iasi was described for the first time in the northeast area of Romania. The appearance of the parasite on specimens harvested in the months of May to October

coincided with high temperatures, maximum of 32⁰C up to 35⁰C in July and August, and the average amount of rainfall of 37.2 l/m². Electronmicroscopic investigations (SEM) on *Lipoptena cervi* parasite revealed the body covered with hair threads, multifaceted eye, and robust claws for anchoring the host body. Histologic examination of the skin revealed a

moderate diffuse hyperkeratosis. The dermis was characterized by infiltrates of inflammatory cells: eosinophilic granulocytes, lymphocytes, macrophages. Blood vessels have an endothelial hypertrophy and inflammatory infiltrates with Manson character.

REFERENCE

- DEHIO, C.; SAUDER, U.; HIESTAND, R. Isolation of *Bartonella schoenbuchensis* from *Lipoptena cervi*, a blood-sucking arthropod causing deer ked dermatitis. *J. Clin. Microbiol.*, v.42, p.5320-5323, 2004.
- HALOS, L.; JAMAL, T.; MILLARD, L. et al. Role of *Hippoboscidae* flies as potential vectors of *Bartonella spp.* infecting wild and domestic ruminants. *Appl. Environ. Microbiol.*; v.70, p.6302-6305, 2004.
- HÄRKÖNEN, S.; LAINE, M.; VORNANEN, M.; REUNALA, T. Deer ked (*Lipoptena cervi*) dermatitis in humans - an increasing nuisance in Finland. *Alces*, v.45, p.73-79, 2009.
- HODŽIĆ, A.; OMERAGIĆ, J.; ALIĆ, A. et al. *Lipoptena cervi* (Diptera: hippoboscidae) in roe deer (*Capreolus capreolus*). *Veterinaria*, v.61, p.17-21, 2012.
- KYNKÄÄNNIEMI, S.M.; KORTET, R.; HÄRKÖNEN, L. et al. Threat of an invasive parasitic fly, the deer ked (*Lipoptena cervi*), to the reindeer (*Rangifer tarandus tarandus*): experimental infection and treatment. *Ann. Zool. Fenn.*, v.47, p.28-36, 2010.
- MADSLIEN, K.; YTREHUS, B.; VIKØREN, T. et al. Hair-loss epizootic in moose (*Alces alces*) associated with massive deer ked (*Lipoptena cervi*) infestation. *J. Wildl. Dis.*, v.47, p.893-906, 2011.
- PAKKONEN, T.; MUSTONEN, A.M.; KÄKELÄ, R. et al. The effects of an abundant ectoparasite, the deer ked (*Lipoptena cervi*), on the health of moose (*Alces alces*) in Finland. *Parasitol. Res.*, v.111, p.1223-1232, 2012.