



Short communication

Oestrosis in Asiatic ibex (*Capra sibirica*): a case report and molecular characterization of larvae



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ABSTRACT

Three third-instar *Oestrus* larvae were found in the frontal sinus of an adult female Asiatic ibex (*Capra sibirica*) in the Tian Shan mountain range, Kyrgyzstan. The larvae were identified as *Oestrus ovis* based on morphology and after sequencing and analyzing a fragment of the cytochrome oxidase I (COI) gene. In light of this identification and the fact that Asiatic ibex and livestock are sympatric in many areas in Central Asia, we discuss the risks of interspecific parasite spillover.

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1. Introduction

The Asiatic ibex (*Capra sibirica*), also called Siberian or Himalayan ibex (Sarasa et al., 2012), is a wild Bovidae species living in the high mountain ranges of Central Asia stretching across parts of Afghanistan, China, India, Kazakhstan, Kyrgyzstan, Mongolia, Pakistan, Russia, Tajikistan and Uzbekistan (Shackleton, 1997). Its current conservation status is considered “Least Concern” by the International Union for Conservation of Nature due to presumed large populations and an unknown population trend (Reading and Shank, 2008).

Several parasite species have been found in the Asiatic ibex: coccidia (Tilc and Hanuskova, 1976), helminths (Danzan and Drozd, 1964; Tilc and Hanuskova, 1976; Kuchboev et al., 2015) and *Sarcoptes scabiei*, the agent of sarcoptic mange (Vyrypaev, 1985). Two nasal bot flies have been reported parasitizing the species: *Oestrus caucasicus* and *O. ovis* (Grunin, 1957; Minar et al., 1985; Colwell et al., 2006). However, these species were identified from larvae phenotypes, a difficult approach, as *Oestrus* larvae belonging to different species can be morphologically very similar (Wetzel and Bauristhene, 1970; Guitton et al., 2001). Molecular characterization is a more accurate method to identify *Oestrus* larvae specimens (Moreno et al., 2015).

The main goal of this study was to determine the identity of *Oestrus* larvae parasitizing a recently dead Asiatic ibex using a molecular characterization approach, and to increase our understanding of parasite spillover risk between livestock and wildlife in Central Asian highlands.

2. Materials and methods

2.1. Collection and morphological identification of larvae

A recently dead (<12 h) Asiatic ibex was found during a routine wildlife survey on May 2015 in the Central Tian Shan mountain range, Kyrgyzstan (UTM coordinates: WGS84 44T 277054 4614515), at an altitude of 3436 m asl. The animal, an adult female aged 7–8 years, showed evidences of having been killed for food as we only found the skin, leg extremities, a partially scavenged digestive tract with some abdominal fat and the skull. Three *Oestrus* third-instar larvae were collected from the frontal sinuses (Fig. 1), washed with normal saline, fixed and transported in 90° ethanol, and stored at 4 °C until analysis. We used morphological features provided in Zumpt (1965) and Wetzel and Bauristhene (1970) as a preliminary identification.

2.2. DNA extraction and molecular analyses

Genomic DNA was extracted with the Insect genomic DNA extraction Kit (Zymo Research). A fragment (689 bp) of the

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Fig. 1. A third-instar oestrid larva collected from the frontal sinus of a freshly dead adult female Siberian ibex (*Capra sibirica*).



Fig. 2. Ventral (left) and dorsal (right) view of a third-instar *Oestrus ovis* larva showing lack of spines in the lateral sides of the anterior segments.

cytochrome oxidase I (COI) was amplified by PCR using primer pairs Ovis-UEA7 and Ovis-UEA10 (Zhang and Hewitt, 1997). Standard PCR reactions were performed with the annealing temperature of 55 °C. PCR amplicons were resolved in ethidium bromide-stained 1% agarose gels; the appropriate band was isolated from the gel with QIAquick gel extraction kit (Qiagen) and cloned in JM109 bacteria using PGEMT-easy vector (Promega). Two positive clones were sequenced in both directions. Sequences were analyzed using the Bioedit program (version 7.0.9.0) (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). GenBank search for sequences was performed with BLASTN 2.6.0 (<https://blast.ncbi.nlm.nih.gov/>) (Zhang et al., 2000).

3. Results

The three larvae fitted the description for *Oestrus ovis*, regarding size, shape of posterior peritremes and dorsal and ventral spinulation (Zumpt, 1965; Wetzel and Bauristhene, 1970) (Fig. 2).

Two COI clones were sequenced. Analysis demonstrated that both clones contained an identical sequence of 689 pb in length (GenBank accession numbers: KY563713 – KY563714). While the mean reported value for interspecies similitude of COI in the Oestri-

nae subfamily is 86.7% (Otranto et al., 2005), the sequence showed a 99% identity with the COI sequences of *Oestrus ovis* available at GenBank and less than 91% identity with the COI sequences of other *Oestrus* species (*Oestrus* sp.) (Moreno et al., 2015). In addition, the identity of this sequence with the COI sequences of other Oestrid species was less than 85%. These results demonstrated that the larvae analyzed belong to the species *Oestrus ovis*.

4. Discussion

Molecular characterization confirms the presence of *Oestrus ovis* in a free-living Asiatic ibex. Molecular data from *Oestrus* species are rare in wildlife in general. Prior to this case there were COI sequences available in Genbank only for the European mouflon (*Ovis orientalis musimon*) and the Iberian ibex (*Capra pyrenaica*) (Moreno et al., 2015). The new DNA sequence is also the first reported for *Oestrus ovis* parasitizing a wild host in Asia.

Although half of the Old World oestrids are specific to a single host species (Price 1980), this is not the case for *Oestrus ovis*. When other susceptible hosts are available gravid females are not strictly host-specific but domestic sheep and goats are thought to be reservoir and a source of infection for wildlife (Colwell, 2001). In the context of co-habitation of livestock and wildlife, the species has been reported to parasitize the argali (*Ovis ammon*), the Alpine ibex (*Capra ibex*), the bighorn sheep (*Ovis canadensis*), the European mouflon, the white-tailed deer (*Odocoileus virginianus*) and other wild and domestic host species (Moreno et al., 1999; Colwell et al., 2006).

The occurrence of *O. ovis* in wild hosts in Central Asia is poorly known. Early records from the middle of the twentieth century reported that in addition to the Asiatic ibex, the parasite was found in three out of 15 examined argali from the Tian Shan mountain range (Grebenuik and Sartbaev, 1995) and in three argali in the Pamir mountain range (Sapozhnikov, 1976). In 2009 *Oestrus ovis* infection (specimen 2009/651-3, Natural History Museum, London) was also noted in six out of 10 hunted adult argali in eastern Pamir of Tajikistan (Ostrowski, pers. comm.).

Domestic sheep and goats are sympatric and serious competitors with ibex and argali across most of the high elevation pastures of Central Asia (Fedosenko and Blank, 2005). In addition gravid *O. ovis* females can fly up to 30 km to find a susceptible host (Anderson, 2006). Hence the resulting risk of spillover of *O. ovis* from livestock to wildlife is potentially high in the region. In contrast to livestock the impact of oestrosis in wild ungulates is largely unknown. In the European mouflon from Spain, Moreno et al. (1999) found that 46 percent of the animals surveyed were infected yet they reported no associated clinical symptoms. However, cases of ataxia, stumbling and accidental falls have been observed recently in argali in Pamirs (Ostrowski, pers. comm.), all symptoms compatible with oestrosis as described in the domestic sheep (Dorchies et al., 2006). Finally it has been proposed that oestrosis may be a predisposing factor for contagious caprine pleuropneumonia (CCPP) (Jagannath et al., 1989), a matter of concern for wildlife in Central Asia in which CCPP may cause significant mortality (Yu et al., 2013).

The results obtained in our study call for more research on the risks posed by *O. ovis* to wild mountain ungulates in Central Asia. In the general context of increasing encroachment of livestock into wild habitats, wild ungulates in the region might be at higher risk for future livestock-born parasitosis.

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