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Ashworthius sidemi Schulz, 1933 (Trichostrongylidae: Haemonchinae) in mountain ecosystems – a potential risk for the Tatra chamois *Rupicapra rupicapra tatrica* (Blahout, 1971/1972)

Paweł Nosal [a], Jerzy Kowal [a,*], Anna Wyrobisz-Papiewska [a], Gabriela Chovancová [b]

[a] Department of Zoology and Animal Welfare, Faculty of Animal Sciences, University of Agriculture in Krakow, Mickiewicza av. 24/28, 30-059, Cracow, Poland
[b] Research Station and Museum of the Tatra National Park, Tatranská Lomnica 66, 059 60, Vysoké Tatry, Slovakia

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ABSTRACT

The Asian native *Ashworthius sidemi* is now in Europe, and several dozen years after its introduction, it is a widespread parasite of all wild cervids. For bovids, the nematode is a significant threat to the European bison (*Bison bonasus*) population and has also been found in mouflon (*Ovis aries musimon*). Our study aimed to assess the risk of infection for the endemic subspecies of northern (Alpine) chamois (*Rupicapra rupicapra*) – the Tatra chamois (*R. r. tatrica*), which has a critically endangered status. We conducted the investigation in the mountainous areas of Slovakia and Poland occupied by Tatra chamois (*R. r. tatrica*), Alpine chamois (*R. r. rupicapra*), red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*). Animals (n = 93) shot during licensed hunting and killed in road accidents (roe deer, red deer), or which had died of natural causes (chamois) were post-mortem examined for the presence of Haemonchinae. The investigation confirmed the expansion of *Ashworthius sidemi* to high mountain regions via Cervidae. *A. sidemi* affected all of the examined roe deer and 90.0% of the red deer. As for the chamois, *A. sidemi* was found in one *R. rupicapra* originating from the Low Tatras, but not in any pure *R. r. tatrica* individuals living in the High and Western Tatras. Our work is the first confirmation of northern chamois infection with this alien and highly pathogenic blood-sucking nematode. Due to the important health hazard of *A. sidemi* infection for the Tatra chamois (*R. r. tatrica*), appropriate measures should be taken to reduce the possibility of parasite transmission between various cervid species living in the Tatra region, as well as the affected population of chamois and the pure Tatra chamois population inhabiting the higher parts of the mountains, constituting their natural habitat.

1. Introduction

Ashworthius sidemi Schulz (1933) is an alien and ecologically invasive parasite species of ruminants, which has been spreading in Europe since the second half of the twentieth century. Its occurrence has gradually increased over recent years, especially in cervids, but also among free roaming wild bovids. While it is a typical parasite of Cervidae and occurs in infections usually not exceeding several hundred specimens, its intensity of infection may reach tens of thousands of specimens and cause pathogenesis, even leading to mortality, for non-specific and new Bovidae hosts (Demiaszkiewicz et al., 2008, 2009). Moreover, a typical infection site in cervids of *A. sidemi* is the abomasum, while in other highly affected ruminant hosts, the nematode also parasitizes the small and large intestines (Demiaszkiewicz et al., 2012). Pathological ashworthiosis-related changes include oedema, hyperaemia and effusion in the gastrointestinal mucosa, leading to chronic diarrhoea, deterioration and cachexia, or the animal's death (Demiaszkiewicz et al., 2009).

The first description of this trichostrongylid worm was made by Schulz (1933) from sika deer (*Cervus nippon* Temmink, 1838) living in farm conditions in the Russian Far East. *A. sidemi* was also discovered in introduced sika deer in former Czechoslovakia (Kotrlá and Kotrlý, 1973) and registered in maral (*Cervus elaphus sibiricus* Severtzov, 1873) introduced from Asia to the European part of Russia (Nazarova and Starodynova, 1974). Therefore, its presence among wild ruminants in several European countries can be explained by the translocation of this parasite with sika deer from Asia (Hoberg, 2010). In France, *A. sidemi* was found in sika deer, fallow deer (*Dama dama* Linnaeus, 1758), roe

* Corresponding author.
E-mail addresses: pawel.nosal@urk.edu.pl (P. Nosal), jerzy.kowal@urk.edu.pl (J. Kowal), Anna.Wyrobisz-Papiewska@urk.edu.pl (A. Wyrobisz-Papiewska).

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deer (*Capreolus capreolus* Linnaeus, 1758) and red deer (*Cervus elaphus* Linnaeus, 1758) (Ferté et al., 2000). In Sweden, it was registered in fallow deer of Hungarian origin (Höglund et al., 2007). In the European part of Russia, apart from sika deer and maral, the worm species was registered in native moose (*Alces alces* Linnaeus, 1758) and roe deer (Nazarova and Starodynova, 1974; Kuznetsov et al., 2018). In Belarus, it was found in European bison (*Bison bonasus* Linnaeus, 1758) (Kochko, 2003) and detected in roe deer in Ukraine (Kuzmina et al., 2010). In Czechoslovakia, apart from sika deer, red deer and mouflon (*Ovis aries musimon* (Pallas, 1811)) have also harboured this parasite (Kotrlý and Kotrlá, 1980).

A. sidemi has been documented as an introduced species in Poland since 1997 (Dróżdż et al., 1998). The parasite was observed for the first time in the country in several European bison in the Bieszczady Mountains (Eastern Carpathians, south-eastern Poland). Following confirmation of ashworthiosis in red deer and roe deer living in this region, Dróżdż et al. (2000) concluded that the origin of *A. sidemi* was local red deer, which brought the parasite from neighbouring Ukraine and Slovakia along the Carpathian ecological corridor. Next, in 2001, the nematode was observed in lowland European bison in Białowieża National Park (Dróżdż et al., 2002). Another documented concentration of ashworthiosis was the Dulowa Primeval Forest in southern Poland, where fallow deer introduced from Hungary were found to be infected (Kowal et al., 2012). Recently, the further and rapid expansion of this nematode has been observed among all Cervidae species living in the country, including moose (Demiaszkiewicz et al., 2013), and the parasite has also been identified for the first time in domestic cattle (*Bos taurus* Linnaeus, 1758) by means of the polymerase chain reaction method (Moskwa et al., 2015).

The Tatra chamois (*Rupicapra rupicapra tatrica* (Blahout, 1971/1972)) is a representative of the Bovidae family and the northernmost subspecies of the northern (Alpine) chamois (*Rupicapra rupicapra* (Linnaeus, 1758)), which is native to the mountainous parts of central and southern Europe and Asia Minor. In the Alps, where the bulk of the northern chamois population is found, the species is relatively secure and consequently assessed as least concern (LC) in the International Union for Conservation of Nature's Red List of Threatened Species (Aulagnier et al., 2008). However, several chamois subspecies qualify as globally threatened and require urgent conservation action, including the Tatra chamois, which is listed as critically endangered (CR).

The Tatra chamois occur in the Tatra Mountains of Poland and Slovakia, living in areas protected by national parks of both countries, i. e. in the High, Belianske and Western Tatras. The population has been declining steadily since the 1960s and had dropped to below 200 individuals by 2002 (Aulagnier et al., 2008). Since then, by strictly regulating tourism and suppressing poaching, the population has started to recover, reaching the highest ever recorded population of 1431 individuals in 2018 (https://tpn.pl/nowosci/kozice-policzone-5, accessed Dec 14, 2020).

In Slovakia, Tatra chamois have also been artificially introduced (30 individuals) to the Low Tatras to create a reserve population (Shackleton et al., 1997) (Fig. 1). However, the Alpine chamois were introduced there for hunting purposes before the Tatra chamois were officially classified as a separate subspecies, and the Low Tatra population of *R. r. tatrica* crossbred with Alpine chamois migrating from the Fatra Mountains and the Slovak Paradise National Park. Therefore, it cannot act as a reserve population for the Tatra chamois because it is no longer pure population. On the contrary, according to Shackleton et al. (1997), the *R. r. rupicapra* introduced to Slovakia should be removed, as they pose a threat to the wild population of *R. r. tatrica* living in the High Tatras, from which they are separated by only a single valley – a distance of about 30 km.

The present study aimed to determine the threat to both populations of chamois living in the Tatras from the alien *Ashworthius sidemi*

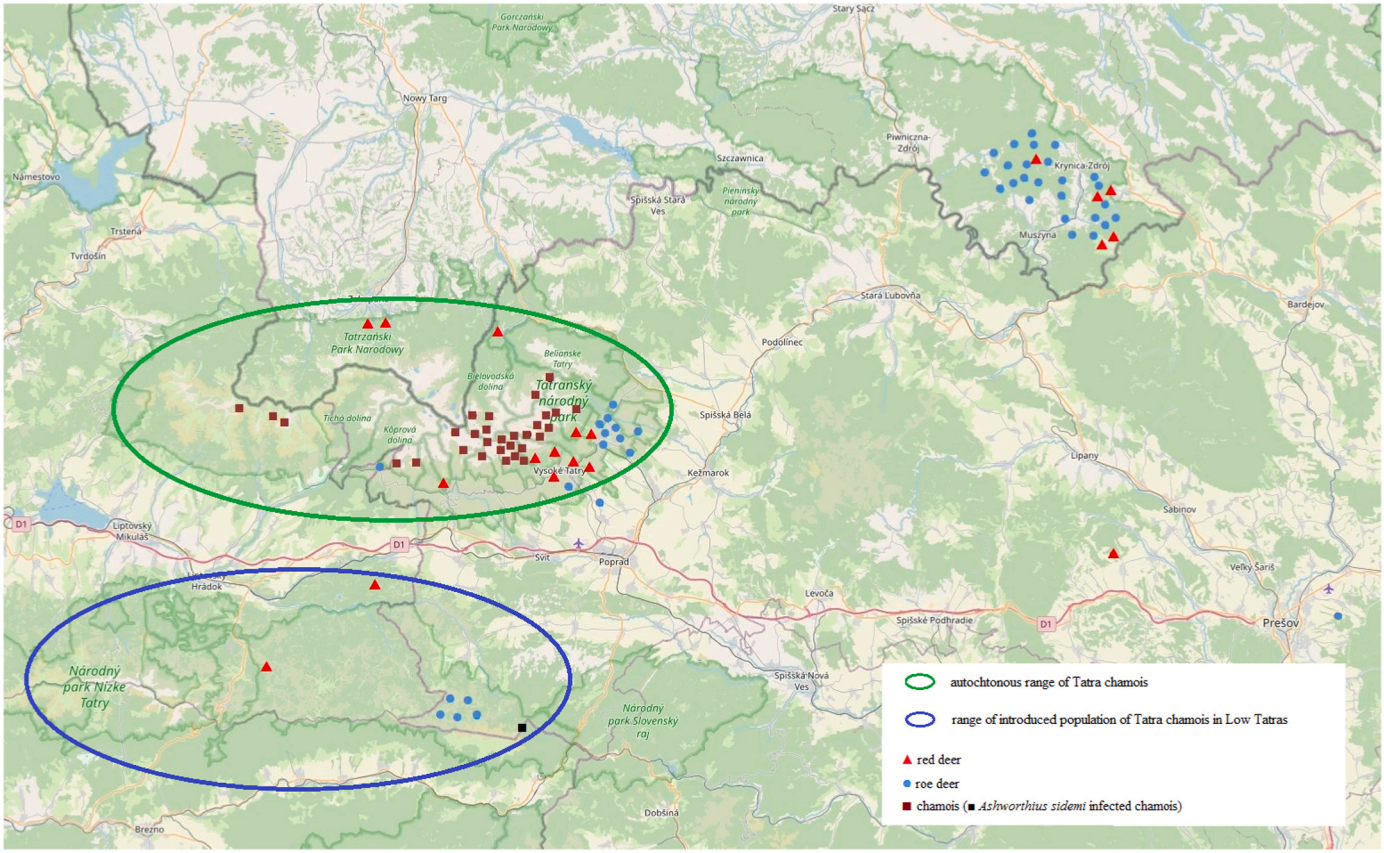

Fig. 1. Map of Tatras showing the geographical origin of the animals included in the study.

nematode. Due to the greater opportunity of contact with cervids of the crossbred population living in the Low Tatras, particular attention was paid to the emergence of infection with this parasite there. If confirmed, then a much higher risk of *A. sidemi* appearance in the second, pure *R. r. tatrica* population living in the High, Belianske and Western Tatras may be indicated.

2. Materials and methods

2.1. Study area and material collection

The study was conducted in the mountainous territory of Tatra National Park (High and Western Tatras; altitude from 800 to 2655 m a.s.l.), Piwniczna Forest District (Beskid Sądecki Mountains, West Carpathians; up to 1114 m a.s.l.), as well as other hunting areas managed by Slovak State Forests, i.e. Liptovky Hradok and Presov (Low Tatras; up to 2043 m a.s.l.). The areas are occupied by Tatra chamois (*R. r. tatrica*; High and Western Tatras), Alpine chamois (*R. r. rupicapra*; Low Tatras), and stable populations of red deer (*C. elaphus*) and roe deer (*C. capreolus*). Animals shot during licensed hunting, killed in road accidents (roe deer, red deer), or that had died a natural death (chamois) constituted the research material.

2.2. Laboratory analysis and identification of worms

Parasitological examinations of the abomasa collected from a total of 93 wild ruminants (Fig. 1, Table 1) were conducted according to a modified Hansen and Perry method (Hansen and Perry, 1994). The whole contents and washings of the mucosa were rinsed over a 250 μ mesh sieve, and then transferred in small portions into Petri dishes to be examined under a stereomicroscope. All isolated nematodes were preserved in 75% ethanol. The Haemonchinae were identified to species on the basis of the morphometric features of the bursa copulatrix, spiculae in males and cuticular lobe around the vulva of females (Lichtenfels et al., 1994; Schulz, 1933).

3. Results

A total of 12067 Haemonchinae specimens, i.e. 12046 of the species *A. sidemi*, and 21 of *Haemonchus contortus*, were collected from the abomasa of infected ruminants. Only one chamois, which was the sole individual originating from the Low Tatras, was infected with *A. sidemi* (Fig. 1). In the abomasum of that animal, 25 nematode specimens were found (Table 1).

No chamois from the High and Western Tatras were affected. Instead, *A. sidemi* was common in cervids from both mountain ranges. All 42 roe deer examined harboured this parasite species, and of 20 red deer, 18 (90.0%) were infected, with a mean intensity of 196 (1–2276) and 210 (2–1756) parasites, respectively. The other Haemonchinae, *H. contortus*, was not found either in chamois or red deer, but it occurred in roe deer with a prevalence of 7.1%, and it co-occurred with *A. sidemi* in two of the three infected animals. The intensity of *H. contortus* infection did not exceed several specimens.

Table 1
Haemonchinae infection in wild ruminants (n = 93) examined post-mortem.

Host species	*Ashworthius sidemi*				*Haemonchus contortus*			
	P (%)	I	R	MA	P (%)	I	R	MA
Rupicapra rupicapra (n = 31)	3.2[a]	25	25	0.8	0.0	–	–	–
Capreolus capreolus (n = 42)	100	196	1–2276	294	7.1	7	4–9	0.5
Cervus elaphus (n = 20)	90.0	210	2–1756	202	0.0	–	–	–

Abbreviations: P – prevalence given as percentage of infected/examined animals; I – mean intensity; R – range; MA – mean abundance.
[a] *A. sidemi* derived from one Alpine/crossbred chamois *Rupicapra rupicapra*; the pure Tatra chamois *R. r. tatrica* were not infected.

4. Discussion

This is the first confirmation of *A. sidemi* infection in the chamois *R. rupicapra*. Although the methodological work of Lehrter et al. (2016) has a statement on the infection of northern chamois with *A. sidemi* – which was later quoted, e.g. by Kuznetsov et al. (2018) – the results of that work (Lehrter et al., 2016) indicate another Haemonchinae species, *H. contortus*, as the one isolated from the examined chamois host.

The generalist nematode *H. contortus* is transmitted in the Alps between populations of domestic and wild ruminants, and is commonly found in northern chamois (Citterio et al., 2006; Cerutti et al., 2010). However, the nematode was never found in chamois living in the Tatras, and the current study also failed to confirm the presence of *H. contortus* in Tatra chamois. Instead, the presence of *A. sidemi* was revealed.

In the autochtonous range of *R. r. tatrica* (Fig. 1) on the Slovak side of the Tatras – although the presence of *H. contortus* together with *Teladorsagia circumcincta* was confirmed in sheep (Mituch 1974) – the grazing of domestic animals is not allowed, whereas on the Polish side, cultural grazing of local sheep breeds is practiced on the mountain pastures of the Tatra National Park. The herds of sheep from this region of Poland are confirmed to be infected by *H. contortus*, *T. circumcincta*, and a dozen nematode species from the genera of *Trichostrongylus*, *Cooperia*, *Nematodirus*, *Aonchotheca*, *Chabertia*, *Oesophagostomum*, *Trichuris* and *Skrjabinema* (Nosal et al., 2015). Apart from *Marshallagia* species, *T. circumcincta* was also noted in the Tatra chamois (Kowal et al., 2017). Fortunately, *H. contortus* seems to be weakly resistant to high mountain conditions (Citterio and Lanfranchi, 2006), and northern chamois may also be able to avoid pastures contaminated with sheep dung (Fankhauser et al., 2008). In the present research, we found cervids to be highly infected with ashworthiosis – both roe deer and red deer, while only roe deer were found to have *H. contortus* specimens. The red deer covers long distances, while the roe deer does not leave its home range and can be a local source of ashworthosis for other ruminant species, including domestic ones. Thus far, *A. sidemi* has been found in the high Tatras only in red deer (Mituch et al., 1992), while our current research has also confirmed its presence in roe deer. The nematode is considered typical for the Cervinae subfamily but ecologically invasive, and all Cervidae and Bovidae species can become hosts. This was the case of the European bison, the introduction of which to the Bieszczady Mountains has considerably changed this bovid's nematode fauna, with the adoption of *A. sidemi* and loss of *H. contortus* (Dróżdż et al., 2002). At the same time, even all the red and roe deer examined were free of *H. contortus*, and only *A. sidemi* was widespread. Double infections of *Ashworthius* and *Haemonchus* were not found in the roe deer studied by Ferté et al. (2000), and only *A. sidemi* occurred in the red deer – similarly to our findings. In a study conducted by Demiaszkiewicz et al. (2016) on red deer from the Lower Silesian Wilderness in south-western Poland, only *A. sidemi* was found, albeit some European authors (Dróżdż, 1966; Kotrlý and Kotrlá, 1980) declare the presence of *H. contortus* in this host species. On the other hand, *H. contortus* was observed as a co-parasite of *A. sidemi* in roe deer in Ukraine (Kuzmina et al., 2010) and in France (Lehrter et al., 2016) – although here, only a few individuals were found, which was also the case in our present investigation.

A. sidemi, as an alien and ecologically invasive parasite species of wild ruminants, is spreading extremely quickly across Poland (Demiaszkiewicz et al., 2017). The present work confirms its spread also in high mountain regions. An important issue associated with the lower risk of *R. r. tatrica* acquiring *A. sidemi* infections may only be the fact that while Alpine chamois normally descend below the forest border of the mountains in winter, and the animals occur from 500 m to 3100 m a.s.l. in the Alps (Aulagnier et al., 2008), the Tatra chamois do so sporadically, ranging from 1200 m upwards – to 2630 m a.s.l. (Krištofík and Danko,

2012).

However, roe deer in the Tatras reach a height of about 1660 m a.s.l. and red deer are found up to a height of 1800–2000 m a.s.l. (Krištofík and Danko, 2012). Further, on the Slovak side of the Tatras, feeding racks for animals are provided at an altitude of 900 m a.s.l. In severe winters, when chamois can descend to such racks, and during the red deer rut – when the cervids climb to high altitudes – the risk of infection is highest. Special attention should also be paid to minimising likely contact between the pure Tatra chamois and the other population from the Low Tatras, which have already been affected by this highly pathogenic parasite. The latest data show that *A. sidemi* also has a very negative effect on the activity of the digestive system of this host species, which may result in weight loss of the infected animals (Vadlejch et al., 2019).

4.1. Conclusions

Hopefully, it seems that no pure *R. r. tatrica*, a critically endangered subspecies of *R. rupicapra*, has acquired ashworthiosis, and this has only occurred in the chamois crossbred population from the Low Tatras. Nevertheless, the risk of *A. sidemi* appearing in the indigenous Tatra chamois is presently very high and requires constant monitoring. It is particularly important to take appropriate countermeasures to reduce the possibility of parasite transmission between the cervids and chamois living in the High, Belianske and Western Tatras.

Ethical statement

The study was performed in accordance with the law regulations in Poland and Slovakia.

Declaration of competing interest

The authors declare that they have no conflict of interests.

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