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CASE REPORT

First detection of *Ehrlichia* cf. *ewingii* in a reindeer (*Rangifer tarandus*) in Hungary

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ABSTRACT

This the first report on the presence of *Ehrlichia* cf. *ewingii* in a reindeer (*Rangifer tarandus*) in Hungary. The animal started to show apathy and loss of appetite and subsequently died shortly after being placed in an enclosure with dense vegetation, following a quarantine period. During the diagnostic necropsy, a large volume of reddish pericardial and thoracic effusion was observed resembling heartwater disease. *Ehrlichia* cf. *ewingii* was detected from the thoracic effusion by PCR and sequencing. The selenium content of multiple organs (liver, kidney, heart and skeletal muscles) were measured, but no increase was found. PCR tests for the detection of adenoviruses from the liver, lungs and spleen were negative. The routine bacteriological culture from the liver did not yield specific results either. To establish a causal relationship between the presence of *E.* cf. *ewingii* and the animal's death, further investigations are necessary.

KEYWORDS

Ehrlichia ewingii, *Rangifer tarandus*

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INTRODUCTION

The reindeer (*Rangifer tarandus*) lives in the Northern Hemisphere, including Northern Russia, Scandinavia, Alaska and Northern Canada. Due to varying climate conditions,

multiple ecotypes have evolved (Heggberget et al., 2002; Tryland and Kutz, 2018). The reindeer was domesticated and is now used for multiple purposes, such as a draft animal, while its milk and meat are important food sources in the Northern Hemisphere (Tryland and Kutz, 2018). Both bulls and cows have antlers. Their body weight ranges between 60 and 320 kg, with bulls being heavier and more robust than cows. They migrate in large herds, a phenomenon that is especially common in the winter (Heggberget et al., 2002). Their feeding habits vary seasonally, as they eat grass, other herbaceous plants and the leaves of trees and shrubs during summertime while in the winter, they scrape dried plants from under the snow. During this time, the bark of younger trees and lichens are the most common food sources of this animal species (Heggberget et al., 2002; Tryland and Kutz, 2018).

Species from the genus *Ehrlichia* are known to cause diseases in mammals. For this to occur, specific tick species are required as vectors. In ruminants, such as cattle, sheep, goat and wild ruminants, *Ehrlichia* (*Cowdria*) *ruminantium* causes heartwater disease (Yunker, 1996). As the name of the disease suggests, a large volume of effusion accumulates in the pericardium and hydrothorax develops. In the lungs, marked oedema occurs (Prozesky, 1987; Yunker, 1996).

The human monocytic ehrlichiosis is caused by *Ehrlichia chaffeensis*, which can also infect dogs and ruminants (Ayan et al., 2024; Dugan et al., 2000; Paddock and Childs, 2003; Wang et al., 2021). *Ehrlichia ewingii* causes canine granulocytic ehrlichiosis and presents a similar disease profile in humans (Qurollo et al., 2019). This pathogen has been detected in white-tailed deer (*Odocoileus virginianus*) (Yabsley et al., 2002). A proven vector tick species is *Amblyomma americanum,* but the DNA of the pathogen has been found in other ticks, e.g. *Dermacentor variabilis* and *Rhipicephalus sanguineus* (Sykes, 2014; Yabsley et al., 2002). However, *E. ewingii* is also indigenous to Africa, where *A. americanum* does not occur. On the other hand, in Africa *E. ewingii* was detected in *R. sanguineus* s.l. ticks for which a vector role was postulated (Ndip et al., 2007). This may have relevance to the study area in Hungary where *R. sanguineus* emerged recently (Hornok et al., 2020).

According to our current knowledge, the native European tick fauna, including *Ixodes ricinus*, *Ixodes persulcatus*, *Ixodes canisuga*, *Dermacentor marginatus* and *R. sanguineus* s.l., are reported to be vectors for *Ehrlichia* species (Estrada-Peña et al., 2018; Zhang et al., 2023). In nature, rodents can serve as reservoir hosts for *Ehrlichia* species, allowing ticks to transmit the disease transstadially to humans and ruminants (Telford et al., 1996).

In a survey conducted using blood samples from roe deer, 58% seropositivity against *Anaplasma phagocytophilum* was detected (Alberdi et al., 2000). Granulocytic anaplasmosis has been reported in a roe deer (*Capreolus capreolus*) calf from Norway (Stuen et al., 2001). In a captive reindeer, following *I. ricinus* infestation, wasting, anaemia and haemoglobinuria were observed. In the mentioned animal, *A. phagocytophilum* and *Babesia divergens* were detected (Romanos and Maillard, 2020). In a Mongolian reindeer herd, an 80% prevalence of *Anaplasma ovis* was observed (Haigh et al., 2008).

In the available literature, there is no record of death caused by *Ehrlichia* sp. in reindeer. However, it is known that other vector-borne pathogens cause a more severe disease in reindeer than in other European cervids originating from warmer climates (Sánchez Romano et al., 2019). For example, the mosquito-borne *Setaria tundra*, which causes severe peritonitis and perihepatitis in reindeer, is asymptomatic in roe deer, even in cases of high infestation intensity (Čurlík et al., 2023; Haider et al., 2018).

MATERIALS AND METHODS

In July 2024, a cadaver of an adult reindeer bull was submitted to the Department of Exotic Animal, Wildlife, Fish and Honeybee Medicine (University of Veterinary Medicine, Budapest, Hungary) from a Hungarian zoo (data not shown) for diagnostic necropsy.

The reindeer was released from quarantine to its enclosure ten days prior to its death. One day before that, the animal was apathetic and had no appetite.

The necropsy of the reindeer was conducted following the standard protocols for ruminant dissection (Jubb et al., 2012). For histological examinations, tissue samples from the animal's organs (spleen, liver, kidney, heart) were fixed in a 10% formaldehyde solution and then sectioned after embedding in paraffin. Finally, they were stained with haematoxylin-eosin.

A blood sample was taken from the heart of the animal, from which a blood smear was made. After staining with Giemsa solution, it was examined under a light microscope using 1.000× magnification (Olympus CX21 Microscope, New York Microscope Company, Hicksville, NY 11801, United States).

From the liver, bacterial cultures were made on 5% sheep blood and Drigalski lactose agar for 24 h at 37 °C under aerobic conditions.

Samples from the liver, kidney, heart and skeletal muscle were sent to the laboratory of the Department of Animal Hygiene, Herd Health and Mobile Clinic (University of Veterinary Medicine, Budapest, Hungary) to determine their selenium content. For that, 2 × 0.5-g samples from each tissue were taken. After adding 5 mL of concentrated nitric acid and 5 mL of 30% hydrogen peroxide, the samples were disrupted in a microwave digestor. Then the samples were filled up to 25 mL with ultrapure water, followed by a five-fold dilution, and after the addition of the internal standard solution, the element concentrations were determined by atomic spectroscopy (Nexion 2000 ICP-MS, Perkin Elmer Inc., USA).

The DNA extraction was conducted from the thoracic effusion collected from the cadaver, for the purpose of *Ehrlichia* detection. This process was carried out by using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

A conventional PCR was used with the primers EHR-16sD 5′- GGT ACC YAC AGA AGA AGT CC – 3′ and EHR-16sR 5′ - TAG CAC TCA TCG TTT ACA GC - 3′ (Brown et al., 2001) to amplify a 350 bp long fragment of the Anaplasmataceae 16S rRNA gene. 5 µL of extracted DNA were added to 20 µL of reaction mixture containing 1.0 U HotStar Taq Plus DNA Polymerase ($5U^{*}\mu L^{-1}$), 0.5 µL dNTP Mix (10 mM), 0.5 µL of each primer (50 µM), 2.5 µL of 10× Coral Load PCR buffer (15 mM MgCl2 included), and 15.8 µL DW. An initial denaturation step at 95 °C for 10 min was followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s. Final extension was performed at 72 °C for 5 min then kept at 40 °C. DNA of Anaplasmataceae (*Anaplasma bovis* and *Ehrlichia canis*) served as a positive control. PCR products were electrophoresed in 1.5% agarose gel (100 V, 50 min), stained with ethidium-bromide and visualized under ultraviolet light.

For each PCR reaction, negative and positive control samples were also run. The positive controls consistently yielded positive results, while the negative controls remained negative in all cases. The purification and sequencing of the products were carried out by Eurofins Biomi Ltd. (Gödöllő, Hungary). The quality checking and trimming of sequences were performed using the BioEdit program. Subsequently, the sequences were aligned with those in GenBank using the nucleotide BLASTN program (https://blast.ncbi.nlm.nih.gov). The 305 base pair sequence obtained during the study has been submitted to the GenBank database under the following accession number: PQ609091.

The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei, 1987). The optimal tree is shown (Fig. 4). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown below the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei et al., 2000) and are in the units of the number of base differences per site. This analysis involved 36 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 305 positions in the final dataset. The evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

RESULTS

Pathological findings

The reindeer was in good body condition, with an adequate amount of adipose tissue visible in the fat stores. The fur, skin, external body orifices and extremities showed no pathological changes.

Upon opening the thoracic cavity, approximately 2 L of dark red, watery transparent fluid was drained from the thoracic cavity (Fig. 1). The lungs were slightly enlarged showing interlobular emphysema and a mottled dark red to light red colour. Beneath the visceral pleura, a noticeable thickening of the septal system was observed (Fig. 2). Approximately 40 mL of dark red, watery transparent fluid was detected in the pericardium. Petechial and suffusion haemorrhages were evident in the epicardium (Fig. 3). The heart was of typical shape and size, with the coronary grooves well-embedded in fat, showing both punctate and confluent haemorrhages (Fig. 3). The myocardium, ventricular chambers and atrioventricular valves showed no pathological changes. We found a normal amount of subcutaneous and visceral adipose tissue, which indicated an adequate nutritional status.

Histopathological and routine bacteriological findings

Due to autolysis, histopathological analyses from the organs (lung, liver, kidney) did not yield interpretable or relevant

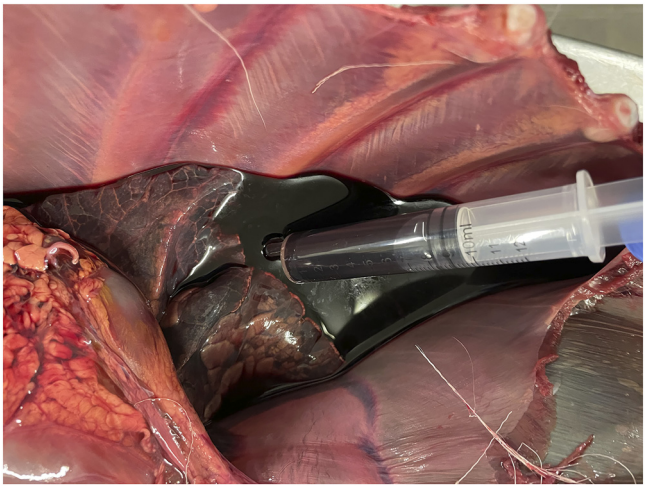

Fig. 1. Large amount of dark red, watery transparent fluid in the thoracic cavity

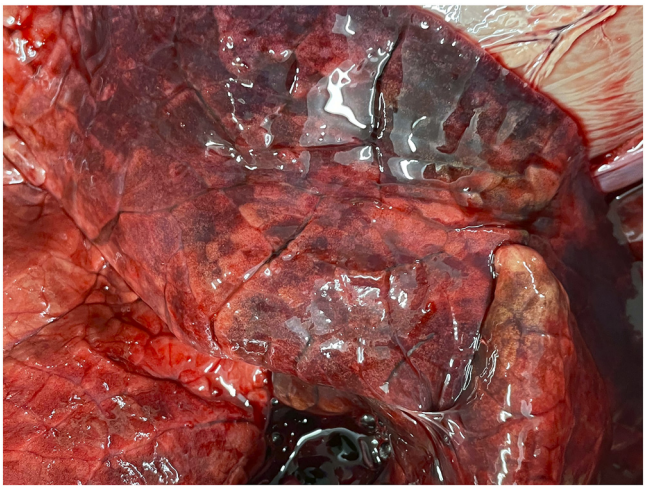

Fig. 2. Interlobular oedema in the lung

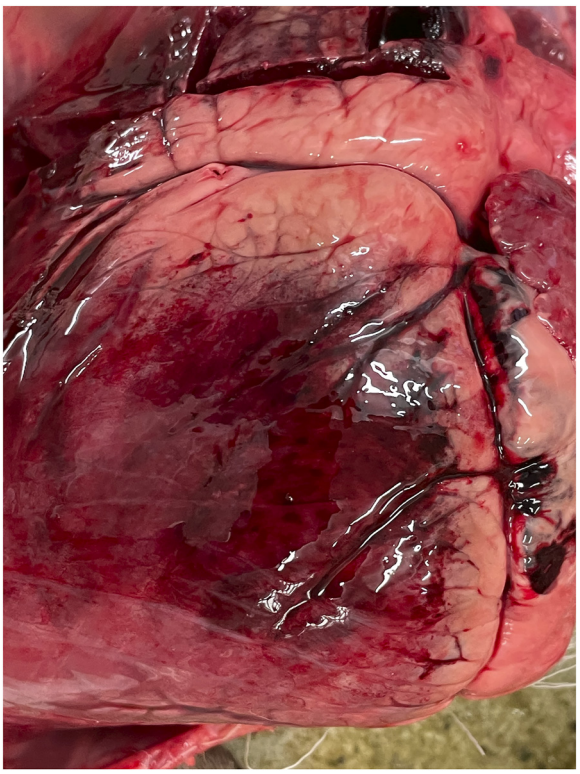

Fig. 3. Petechial haemorrhages and suffusions in the epicardium of the heart

results. In the Giemsa-stained blood smear prepared from the heart blood, a few blood cell forms were observable and numerous bacterial bodies with heterogeneous morphology were visible throughout the smear.

During the bacteriological examination of the liver, no pathogenic bacteria were identified.

Selenium content of the organs

The results of our analysis regarding the selenium content in the organs of the reindeer cadaver are enlisted in Table 1.

Table 1. Selenium content in samples of reindeer heart, skeletal muscle, liver and kidney

Organs	Measured selenium mean (mg^*kg^{-1} wet weight)	Reindeer selenium references (mg^*kg^{-1} wet weight) (Aastrup et al., 2000)
Liver	0.47	0.085–0.984
Kidney	0.89	No data
Heart	0.28	No data
Skeletal muscle	0.20	0.030–0.252

RESULTS OF MOLECULAR AND PHYLOGENETIC ANALYSES

The PCR analysis aimed at the detection of adenovirus DNA from lung, spleen, and liver led to a negative result.

The PCR analysis aimed at detecting the *16S* rRNA of the Anaplasmataceae family yielded a positive result. After sequencing the amplified gene fragment, it was found to be 100% identical to *E. ewingii* and other, yet unvalid *Ehrlichia* species sequences available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

According to our phylogenetic analysis, the sequence we examined (*Ehrlichia* cf. (confer) *ewingii*, PQ609091) clustered with other *E. ewingii* sequences found in GenBank database into a well-defined clade, distinct from all known and valid *Ehrlichia* species, as well as from the species *Neorickettsia risticii* and *N. sennetsu* that were previously classified as *Ehrlichia* (Fig. 4).

The reindeer examined in this study died due to acute fluid accumulation altering the pressure conditions in the thoracic cavity and pericardium — i.e., due to hydrothorax and hydropericardium. In small ruminants prevalent in temperate climates, *Ehrlichia* species primarily cause asymptomatic infections. In our case, we suspect that the infection caused an acute and fatal disease in a reindeer originating from the tundra – an area where the tick vectors are absent, and where reindeer are therefore typically isolated from this pathogen.

Based on the results of the necropsy, selenium toxicity was also suspected, due to haemorrhagic cardiac lesions, as it was previously reported in horses (Desta et al., 2011) and in the European hare (*Lepus europaeus*) (Orosi et al., 2017). However, in the case of the reindeer, the measured values did not reach the toxic threshold. Selenium toxicity has been documented in white-tailed deer, with values between 2.7 and 8.97 mg^*kg^{-1} wet weight (Al-Dissi et al., 2011).

Based on literature data, ehrlichiosis in sheep and goats can be effectively managed with the administration of tetracycline and the infection can be controlled with preventive treatments against ectoparasites (Yunker, 1996).

According to the best of our knowledge, this is the first time that *Ehrlichia* cf. *ewingii* was detected in a reindeer that succumbed to an acute, fatal disease. To establish a causal relationship between the presence of this bacterium and the animal's death, further investigations are necessary, preferably through the identification of new cases, detailed histopathological examination of the organs of deceased animals and monitoring live individuals for the presence of *E. ewingii*. Unfortunately, the entire population we studied perished shortly after our investigation. Despite this, our report highlights the potential impacts of climate change, such as the northward spread of vectors and vector-borne diseases in the Northern hemisphere, where many indigenous species have historically lived unaffected due to cold continental climate. When these populations encounter such pathogens as *Ehrlichia* species, they may respond with high mortality. According to our current knowledge, the only proven, competent vector of *E. ewingii* is *A. americanum*, but the DNA of this pathogen has also been detected in other tick species, e.g., *D. variabilis* and *R. sanguineus* s.l.,

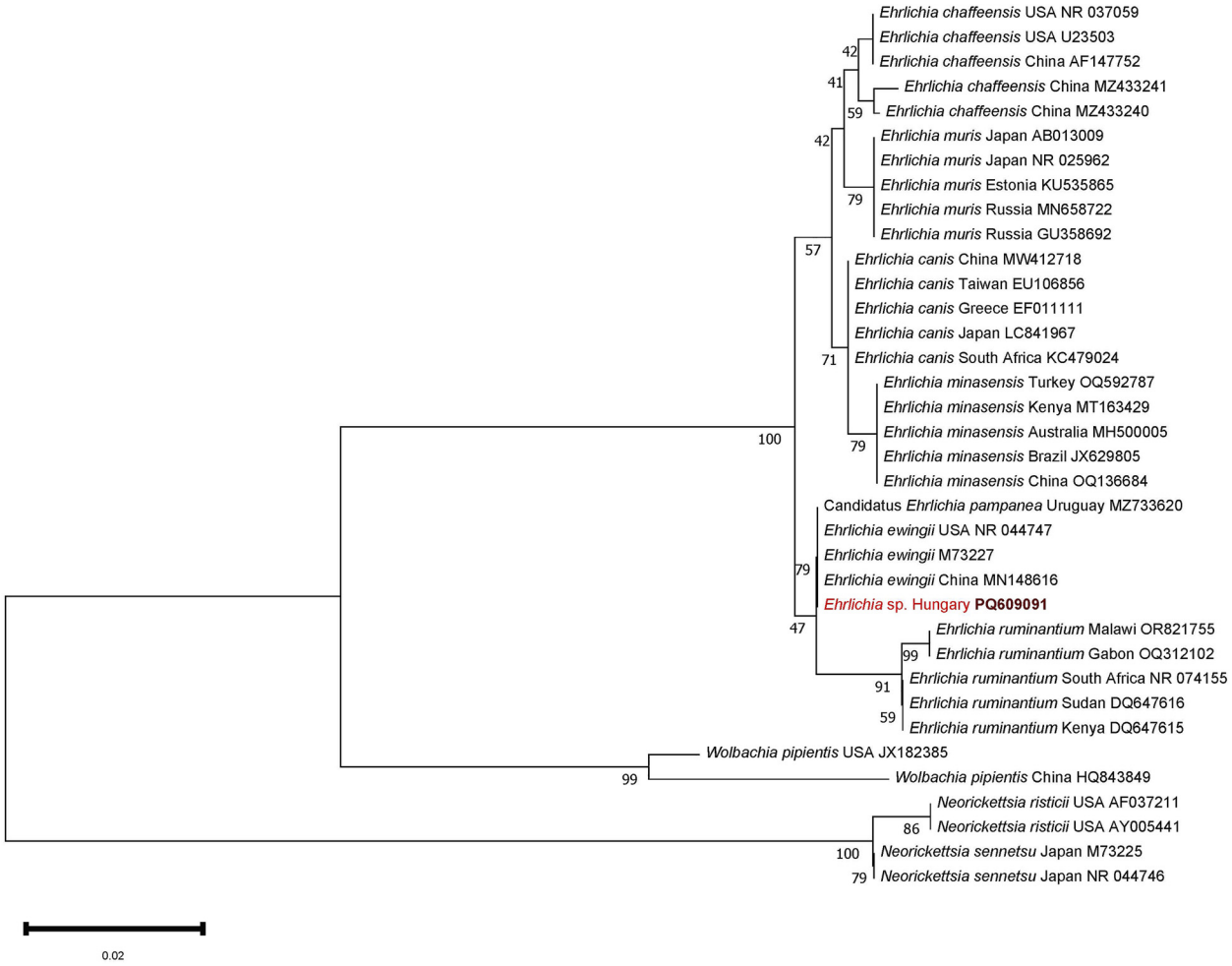

Fig. 4. Phylogenetic tree depicting the *Ehrlichia* cf. *ewingii* sequence from the current study (PQ609091) with other *Ehrlichia* species. For outgroups, *Wolbachia* and *Neorickettsia* species were used

with the latter two suspected to be potential vectors in Africa. While the latter group is known to occur in Hungary, the vector species of *E.* cf. *ewingii* in the study region remains to be identified.

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