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Emerging risk notice: first report of a clinical case of equine theileriosis in Romania

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Summary

The haemoprotozoan parasites *Theileria equi, T. haneyi and Babesia caballi* are the causative agents of equine piroplasmosis. The only effective medication for chemosterilization of *T. equi* is imidocarb dipropionate, which has toxic side-effects in equines, and resistant strains have been reported. We now describe a clinical case of infection with *T. equi* in a horse from Romania. An 18-year-old stallion with an extended travel history presented with weight loss, cough and light colic. Clinical examination revealed fever, tachycardia, tachypnoea and dyspnoea. Mucous membranes were initially anaemic/subicteric, then progressed towards frank icterus. A suspected diagnosis of equine piroplasmosis was made based on clinical signs. A peripheral blood smear was examined and a venous blood sample tested by nested PCR and sequencing. The sequence had 100 % identity to numerous *T. equi* isolates of genotype E. The horse was pretreated with butylscopolamine, followed by two doses of imidocarb dipropionate. It showed clinical improvement within 24 hours and complete remission of symptoms after the end of treatment, although it was still PCR-positive 3 months later. This represents the first confirmed clinical case of theileriosis in a horse from Romania.

Abbreviations: BN = Bistrița-Năsăud County; CJ = Cluj County; EP = equine piroplasmosis; HR = heart rate; RR = respiratory rate; SV = Suceava county

Zusammenfassung

Ein neu auftretendes Risiko: Erstbeschreibung eines Falles von klinischer Theileriose bei einem Pferd in Rumänien

Die Hämoprotozoenparasiten *Theileria equi, T. haneyi* und *Babesia caballi* sind die Erreger der Piroplasmose bei Pferden. Das einzige wirksame Medikament zur Chemosterilisierung von *T. equi* ist Imidocarbdipropionat, das bei Pferden toxische Nebenwirkungen hat, und es wurden schon resistente Stämme beschrieben. In diesem Artikel wird eine klinische Infektion mit *T. equi* bei einem Pferd aus Rumänien beschrieben. Ein 18-jähriger Hengst mit einer langen Reisegeschichte wurde mit Gewichtsverlust, Husten und leichten Koliken vorgestellt. Die klinische Untersuchung ergab Fieber, Tachykardie, Tachypnoe und Dyspnoe. Die Schleimhäute waren zunächst anämisch/subikterisch und entwickelten sich dann zu deutlich ikterisch. Aufgrund der klinischen Symptome wurde die Verdachtsdiagnose equine Piroplasmose gestellt. Ein Ausstrich aus peripherem Blut wurde untersucht, und eine venöse Blutprobe wurde mittels nested PCR und Sequenzierung getestet. Die erhaltene Sequenz wies eine 100%ige Identität mit zahlreichen *T. equi*-Isolaten vom Genotyp E auf. Das Pferd wurde mit Butylscopolamin vorbehandelt, gefolgt von zwei Dosen Imidocarbdipropionat, wobei sich der klinische Zustand innerhalb von 24 Stunden verbesserte und die Symptome nach Beendigung der Behandlung

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vollständig verschwanden. Das Pferd war aber drei Monate später immer noch PCR-positiv. Es handelt sich um den ersten bestätigten klinischen Fall von Theileriose bei einem Pferd aus Rumänien.

Introduction

The haemoprotozoan parasites *Theileria equi, T. haneyi* and *Babesia caballi* are the causative agents of equine piroplasmosis (EP), a tick-borne disease affecting horses, mules, donkeys and zebras (Knowles et al. 2018; Elsawy et al. 2021). The parasites are localized in the erythrocytes and lymphocytes (Rothschild 2013) and can cause asymptomatic disease or severe illness with important clinical and economic consequences (Scoles & Ueti 2015). The disease is endemic in many parts of the world, including countries in Europe, and its occurrence is dependent on the presence of competent tick vectors of the genera *Hyalomma, Dermacentor, Rhipicephalus* and possibly *Ixodes* (Scoles & Ueti 2015; Nadal et al. 2022). Parasitaemia differs between the two genera. It is considered low and temporary for *B. caballi* but lasts the entire lifetime of equines for *T. equi*, irrespective of treatment (Schein 1988). This species is also considered to be more pathogenic (Onyiche et al. 2019). In a natural infection of equines by *T. equi*, clinical signs may include acute severe haemolytic anaemia, which results in weakness, haemoglobinuria and even death, with the most severe form represented by the peracute evolution in foals infected vertically (Chhabra et al. 2012). Direct microscopic examination of blood samples for *T. equi* is often unreliable, especially in chronic infections associated with low parasitaemia. Molecular techniques based on PCR assays for specific genes are also available (Torres et al. 2021). At present, the only effective medication for chemosterilization of *T. equi* is imidocarb dipropionate, which has toxic side effects in equines such as severe colic and diarrhoea, with a significant increase in frequency and quantity of defecation as well as faecal water content due to the inhibition of cholinesterase (Kutscha et al. 2012). Resistant strains have been reported - the pharmaceutical does not always achieve complete clearance (Hines et al. 2015; Sears et al. 2020). A variation in response to the classic imidocarb dipropionate protocol has been reported in natural and experimental *T. equi* infections (Grause et al. 2013).

In Romania, there have only been two studies of the seroprevalence of *T. equi* in horses, one in the rural area of the Danube Delta (Gallusová et al. 2014) and more recently one in western Romania (Giubega et al. 2022). In addition, one horse born in Romania but with an Austrian passport was diagnosed by means of serology in Austria (Dirks et al. 2022).

We now report a clinical case of infection with *T. equi* in a horse that was moved to different places and its recovery after treatment with imidocarb dipropionate.

Case presentation

An 18-year-old stallion (Koninklijk Warmbloed Paard Nederland breed) from a herd in Pojorâta (Suceava county - SV) was presented for a fertility check in Sirioara (Bistrița-Năsăud County - BN) and then transferred to Cluj-Napoca (Cluj County - CJ), where it was housed in a stable with eight other horses. The horses had access to pastures and at night were housed inside on straw bedding. On return from BN (November 2022), the patient started to lose weight and the owner reported that it was lethargic. One month later, the horse became anorexic and apathetic and had a light colic episode accompanied by ephidrosis and coughing. Clinical examination revealed an inner body temperature of 40 °C, heart rate (HR) of 60 bpm with a weak, bounding pulse, dyspnoea and a respiratory rate (RR) of 24. While mucous membranes were initially anaemic/subicteric, they progressed towards frank icterus (Fig. 1) and so the field veterinarian made a suspected diagnosis of piroplasmosis. The horse was pre-

Fig. 1: The clinical aspect of the oral mucosa. Note the yellowish appearance of the gums. / Besichtigung der Maulschleimhaut. Man beachte die gelbliche Verfärbung der Schleimhaut.

treated intravenously each time with a 5 ml per 100 kg body weight dose of an n-butylscopolamine and sodium metamizole product, as recommended by the producer (Buscopan® compositum, Boehringer Ingelheim Vetmedica, Germany), followed by two doses of 4 mg/kg imidocarb dipropionate (Imizol®, Intervet) 48 h apart. Symptoms were reduced within 24 h, and there was complete remission after finishing the imidocarb dipropionate course. Before the first treatment, a peripheral blood smear was stained and microscopically examined but was negative for piroplasms. An EDTA blood sample was collected from the jugular vein and genomic DNA was extracted from 200 µl of whole blood using the commercial kit Isolate II Genomic DNA (Meridian Bioscience, London, UK). A nested PCR protocol targeting the 18S rRNA gene of Piroplasms and Hepatozoon spp. was used for screening (Hodžić et al. 2015). The band was excised from the gel, purified using a commercial kit and sequenced bidirectionally at Macrogen Europe (Amsterdam, NL). The sequence (484 bp) had 100 % nucleotide identity to numerous *Theileria equi* isolates (e.g. MT093500, MZ327270, MT767169). A phylogenetic analysis was conducted using MEGA X software (Kumar et al. 2018) with the Maximum Likelihood method and the Tamura 3-parameter model (Tamura 1992). It included 13 nucleotide sequences: the one obtained during the present study, 11 sequences of *T. equi* (known genotype) retrieved from GenBank® and one sequence of *T. parva* as outgroup. The sequences were aligned using the MUSCLE algorithm and a discrete Gamma distribution was used to model evolutionary rate differences among sites. Our sequence clustered together with other genotype E isolates from Europe and Asia (Fig. 2).

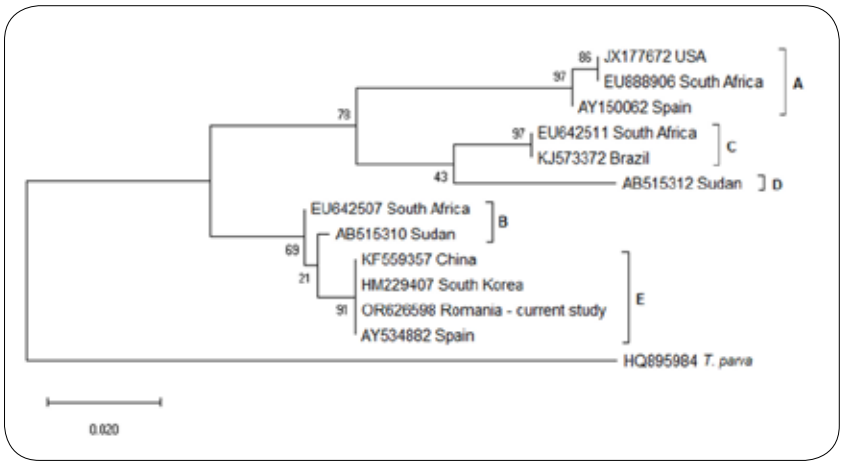

Fig. 2: Bootstrap Maximum Likelihood consensus tree inferred from 1000 replicates. The tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 13 nucleotide sequences, with a total of 486 positions in the final dataset. / Bootstrap Maximum Likelihood Konsensbaum, abgeleitet aus 1000 Wiederholungen. Dargestellt ist der Baum mit der höchsten Log-Likelihood. Der Prozentsatz der Bäume, in denen sich die zugehörigen Taxa zusammengefunden haben, ist neben den Zweigen angegeben. Der Baum ist maßstabsgetreu gezeichnet, wobei die Länge der Zweige in der Anzahl der Substitutionen pro Stelle gemessen wird. Die Analyse umfasste 13 Nukleotidsequenzen mit insgesamt 486 Positionen im endgültigen Datensatz.

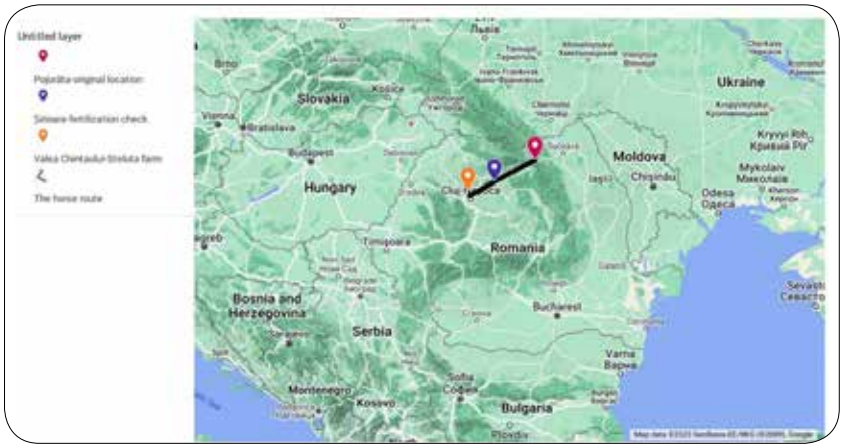

Fig. 3: The patient's route during clinical development / Die Route des Patienten während der Entwicklung der Erkrankung

The other animals (n=8) from the same group presented various clinical signs: icterus, dry cough, fever and slow recovery after exercise but no weight loss. Unfortunately, there were no other investigations as the responsible veterinarian clinically diagnosed the horses as having a piroplasm infection. All animals were treated in the same way as the present case. The diagnosis was confirmed by the remission of symptoms after the first dose of imidocarb dipropionate.

Horses on the farm did not receive any topic prophylactic antiparasitic treatments except for a citronella-based spray from time to time in the hot season. Routine deworming with oral ivermectin (Ecvirom®, Romvac, Ilfov, Romania) was administered every three months. The owner declared that he found ticks attached but not in large numbers. Every time ticks were observed, they were immediately removed. The ticks were not collected and their identification was not possible. After the remission of symptoms, the owner decided to return the horse to its original location in SV (Fig. 3). A check-up three months post-treatment revealed a clinically healthy patient, although the PCR examination yielded similar positive results to the first diagnosis, so the patient was considered to be a carrier of *T. equi*.

Discussion

The present report, along with the limited published data, shows that the prevalence and distribution of equine piroplasms in Romania are insufficiently known. There have been two studies of the prevalence in domestic horses from eastern Romania and semi-feral horses from Danube Delta (Gallusová et al. 2014; Giubega et al. 2022). However, our sequence could not be compared to the previous isolates from Romania (Gallusová et al. 2014), as we amplified a different segment of the gene. There is a need for further nationwide studies to determine the real and local prevalence values and to establish the dominant parasite responsible for equine piroplasmosis in the country, which may have an endemic evolution for *Theileria*, as in other European countries (Camacho et al. 2005; Rocafort-Ferrer et al. 2022). The clinical signs were suggestive of piroplasmosis infection: hyperthermia, tachycardia and tachypnoea accompanied by dyspnoea and icterus. Leading up to this episode of acute clinical presentation, there were several weeks of reduced exercise tolerance and weight loss. As anaemia is progressive in animals infected with *T. equi* and is accompanied by thrombocytopenia and hyperbilirubinaemia during parasitaemia peaks, the clinical manifestation is consistent with other reports (De Waal & Heerden 2004). *Theileria equi* infection frequently evolves as a subclinical infection and presents with a decreased haematocrit and platelet count and haemolytic anaemia (Camacho et al. 2005). Pronounced jaundice is more common in *B. caballi* infections than in infection with *Theileria* spp., which usually appears to evolve with pale mucous membranes (Dirks et al. 2021); nonetheless, icterus became visible 5 days after the first signs in the present case. Coughing is not necessarily a common symptom of *T. equi* infection in horses but is consistently reported in other *Theileria*-infected species (Almazán et al. 2022) and it appeared in our case. Piroplasm infections can be maintained and disseminated by asymptomatic carrier animals, especially during national and international movements. In the present case, the exact location of infection cannot be determined, as the stallion was repeatedly moved during a short time period. It is possible that the acute disease was triggered by stress during transportation or by environmental changes, although this theory is not supported by recent findings that *T. equi* parasitaemia is not affected by stress conditions (Tirosh-Levy et al. 2020a). A negative test for EP is often required for the exportation of horses (Camino et al. 2020).

The preferred treatment for EP remains imidocarb dipropionate, as was used here. There are specific administration protocols (De Waal & Heerden 2004) for *B. caballi* (2–2.5 mg/kg, once a day i.m., twice at 24-hour intervals) and *T. equi* (4 mg/kg, once a day i.m., four times at 72 h intervals) but the responsible veterinarian used a different protocol that seemed to be clinically effective but did not lead to a parasite-free status. In endemic areas, the use of imidocarb dipropionate does not sufficiently control the disease and additional measures should be taken (Onyiche et al. 2019). An effective option would be to control the activity of tick vectors using specific ectoparasiticide products and technological methods, or to limit the contact between horses and ticks.

Eight species of ticks in the Ixodidae family are considered to be vectors for EP: *Dermacentor reticulatus, D. marginatus, Rhipicephalus bursa, R. sanguineus, R. annulatus, Haemaphysalis marginatum, H. punctata* and *Ixodes ricinus* (Nadal et al. 2022) although the competence of the latter species as a vector for EP is still under discussion. It is widely distributed in Europe and feeds on a wide range of hosts, which could reduce the risk of transmission of piroplasms specific to horses (Nadal et al. 2022). Of the tick vectors, *D. marginatus* seems to have an important role in EP transmission (Scoles & Ueti 2015) and is widely distributed in Romania (Estrada-Peña et al. 2017). In contrast, *D. reticulatus* is more frequent in central and northeastern Romania, including Suceava County, one of the possible places of infection in this case. Other than *I. ricinus*, none of the potential vectors have been reported in Sirioara, Bistrița County, so we conclude that the horse was infected in its original location (Suceava). Suitable vectors are known to be present in Cluj County and the infected horse might have represented an reservoir for the infection of the other animals housed there.

The prevalence of EP is directly dependent on the presence of competent tick vectors and their peak activity periods are dependent on their specific ecology. There are frequent co-infections with *T. equi* and *B. caballi*, presumably linked to the shared vectors (Nadal et al. 2022). In the present case, we cannot exclude co-infection as we did not detect any parasitic forms in the blood smears and the PCR protocol does not identify co-infections, due to the preferential amplification of the dominant DNA template. In Romania, financial considerations mean that most horses that manifest fever or have other signs of infection are tested for EP using general molecular tools and all the positive ones are specifically treated without identifying the piroplasm species by sequencing. Sterilization is not consistently achieved (Hines et al. 2015) but without further testing after treatment the carrier status of treated animals remains unknown. Serology should be used to determine the status of infection in both symptomatic and asymptomatic animals (Giubega et al. 2022). In Romania, equine theileriosis has not attracted much interest from researchers and veterinarians and, while the disease is probably endemic, there is very limited information on serological screening of horses in the country (Gallusová et al. 2014; Giubega et al. 2022; Tirosh-Levy et al. 2020b). As the PCR in the present case was still positive at 3 months post-treatment, the

patient represents an important carrier for *Theileria* and might have been implicated in the suspected outbreak of piroplasmid infection in the last housing in which it had been kept.

This case report underlines the presence of *T. equi* in horses from Romania and highlights the need for further large-scale studies to determine the exact extent of the disease.

Conclusion

T. equi has recently gained significance as an emerging disease in non-endemic countries. Its introduction to areas with low endemicity poses a threat to equine health and to the horse industry. In areas where the disease is not commonly encountered, differential diagnosis and accurate identification by PCR and/or cELISA are very important. It is crucial to distinguish between *T. equi* and *B. caballi* due to the potential for animals to become lifelong carriers of *Theileria* but not of Babesia. PCR-based diagnosis aids in identifying infected animals with clinical disease and can accompany cELISA to enable appropriate management strategies, prevent disease spread and minimize the risk of transmission. This ensures effective control and safeguards the equine population in non-endemic countries and in those where the pathogens are considered to be emerging. There is a need for larger scale studies in countries where the extent and prevalence of EP are not known to obtain a more comprehensive view of *T. equi* epidemiology in Europe.

Fazit für die Praxis:
Die equine Piroplasmose und insbesondere die Infektion mit *Th. equi* wird in Rumänien wahrscheinlich unterschätzt. Die Diagnose erfolgt meist anhand der klinischen Symptomatik ohne Bestätigung durch spezifische Laboruntersuchungen. Da sich die Theileriose in vielen Ländern Europas ausbreitet, oft schwerwiegende Symptome bei Pferden verursacht, und Pferde aus verschiedenen Gründen oft über weite Strecken verbracht werden, sollten die Tiere vor Transporten auf Theileriose untersucht werden. Landesweite Studien zur Bestimmung der Prävalenz des Erregers werden empfohlen.

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Conflict of interest
The authors declare no conflict of interest.

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