

Recovery and confirmation of *Haemonchus contortus* from abomasal contents of roe deer (*Capreolus capreolus*) in Eastern-Hungary (Biharugra): A diagnostic case study

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SUMMARY

Gastrointestinal parasites are ubiquitous. They occur both in wild and domesticated animals. Among such parasites of veterinary importance is the trichostrongyle worms, out of which the *Haemonchus contortus* species is regarded as the most pathogenic one in the small ruminant industry. The occurrence of this parasite in the sheep flock is now very well documented and an established fact in Europe, although the parasite was original of the warmer climatic region. Studies on the cross-transmission of *H. contortus* between the wild and domesticated animals are also on the rise although the question of the direction of transmission is still debated. This is an important area that needs to be addressed as it could potentially contribute indirectly to mitigating anthelmintic resistance. Hungary also has reported its share of the occurrence of the parasite, mainly in the sheep flock and a certain population of roe deer. The study presented here is the preliminary results of a diagnostic case study that confirms the presence of *H. contortus* in wild ruminant deer species that are close to the domesticated sheep population.

Keywords: *Haemonchus contortus*; Roe deer; Hungary; PCR

INTRODUCTION

Ruminant animals can be infected as well as be a source of transmission of many gastro-intestinal nematode (GIN) parasites. A significant amount of research has been conducted on the trichostrongylidae family that includes *Haemonchus contortus*, *Ostertagia ostertagi*, *Teladorsagia circumcincta* and *Trichostrongylus axei* to name a few (Halvarsson et al., 2022) as they are important in causing parasitic gastro-enteritis. Among this family, *H. contortus* is regarded as the most pathogenic and widely studied GIN parasite of small ruminants (Besier et al., 2016; Waller et al., 2005). The pathogenic effect of this parasite is attributed directly to the blood-sucking behaviour of the adult worms residing in the abomasum of the host animals. A severely affected animal could lose up to 30 ml of blood per day as each adult parasite can consume around 30–50 µl of blood per day (Clark et al., 1962; Le Jambre, 1995) thereby causing immense economic losses to the sheep and goat industry (Sutherland and Scott, 2010).

Pasture grazing is still the most widely adopted feeding management method for small ruminant farmers and breeders (Laca Megyesi et al., 2020). Farming system on pastures is also considered to be more eco-friendly, requiring minimal input, with the least animal welfare concerns if the overall health of the animal is well taken care of (Csivincsik et al., 2017). Roe deer (*Capreolus capreolus*) are typically found in lowland pastures to montane forests (Laca Megyesi et al., 2020). This deer species is also important as a hunting and game animal (Nagy and Bencze, 1973) in Hungary, besides being an item for the culinary menu. Although *H. contortus* is generally a parasite of domesticated ruminant animals, the nematode worm is also capable of infecting a large number of artiodactyl

hosts (Hoberg et al., 2004; Lehrter et al., 2016), including roe deer. There are already reports available on the detection of *H. contortus* and other related trichostrongyle worms in roe deer from China, Hungary, Poland, Sweden, Turkey and Ukraine (Bolukbas et al., 2012; Halvarsson et al., 2022; Kuzmina et al., 2010; Nagy et al., 2016; Nilsson, 1971; Pilarczyk et al., 2016; Shen et al., 2017). Hence, the suspicion of cross-infection of GIN parasites between domesticated animals and wild animals sharing pastures and in close proximity to each other is growing increasingly.

On this note, we are presenting a case report of worms, suspected to be of *H. contortus*, recovered from the abomasum of farmed roe deer in the Biharugra, Békés County in Hungary. We present here the results of our morphological analysis as well as molecular diagnosis confirmation of the suspected samples.

MATERIALS AND METHODS

Samples

This case report is part of a larger study that deals with the diagnosis of *H. contortus* in and around Eastern Hungary. The abomasum (n=20) of roe deer was obtained courtesy of the hunters in the Biharugra village in late 2019. Each abomasum was dissected longitudinally in a tray taking care its contents were not wasted; it was then gently washed with clean water and sieved using a mesh cloth and finally examined for worms. The mucosal walls of the abomasum were also thoroughly checked to see if any worms were attached. Worms collected were gently washed in tap water and finally collected in falcon tubes with 10% formalin for other downstream processes. Two groups of worms were made: i) Suspected Group (of *H. contortus*) and ii) Unknown Group.

Microscopic Examination of worm

Each collected worm was observed under a microscope at 10x and 20x magnification using Olympus BX51 microscope at the Parasitology Laboratory of the Doctoral School of Animal Science, University of Debrecen. Morphological identification keys of the parasite were done as given by (Taylor et al., 2015). Photographic records of the specimen were also taken using the cellSense software (Olympus) using the Olympus BX51 microscope mounted with a DP70 camera (Olympus).

Species-specific ITS2 PCR

For species confirmation, the specimen parasites were used to extract DNA and then analysed with ITS2 PCR using the same set of primers as per Redman et al. (2008). The DNA was extracted using E.Z.N.A.[®] Tissue DNA Extraction Systems (Omega Bio-tek) following the manufacturer's instructions. The PCR was performed with a total reaction volume of 12.5 µL in each tube constituting: 2.5 µL 5× GoTaq Green Buffer (Promega), 1.25 µL MgCl₂ 25 mM, 0.25 µL dNTPs 10 mM each (VWR International), 0.25 µL (10 µM) each of forward and reverse primers, 0.06 µL GoTaq Flexi polymerase 5 U/µL (Promega), 6.94 µL of molecular grade water and 1 µL of DNA template. Positive control gDNA was obtained from the

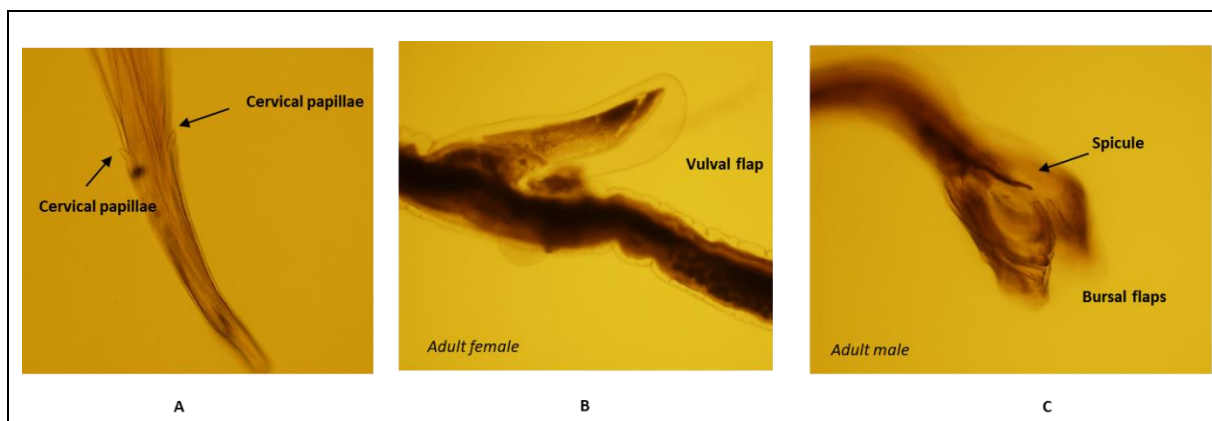
Department of Zoology and Parasitology, University of Veterinary Medicine, Budapest. The thermal profile of the PCR was set up as: denaturation at 94 °C for 2 min; 35 cycles each of 94 °C 30 s, the annealing temperature of 50 °C for 30 s and 72 °C extension for 30 s, with a final extension step of 72 °C 10 min. Results of the amplification were observed using ChemiDoc XRS+ System and Image Lab (BioRad) after gel electrophoresis in 2% agarose gel stained with ethidium bromide. The target amplicon size was 320 bp.

RESULTS AND DISCUSSION

Morphological characteristic

The microscopic analysis result of the specimen worms of the Suspected Group is given in *Figure 1*. The solid arrow shows the cervical papillae (*Figure 1-A*). Trichostrongyle worms are unisexual and thus, male and female worms are usually presented with distinguishing morphological features. The adult *H. contortus* females have a typical vulval flap which is linguiform in shape (*Figure 1-B*). Adult trichostrongyle nematode males have a bursa, which is a modified cuticular appendage at its posterior end. For *H. contortus* males, this bursa has a characteristic hooked dual spicule (*Figure 1-C*). The gross morphology of the worm was reddish and ranged from 2–3 cm in length.

Figure 1: Characteristic morphological features of adult H. contortus. A: head of the worm showing cephalic papillae (arrows); B: adult female with vulval flap; C: adult male with bursal flap and spicules (arrows). 20x magnification



The summary of the key identification criteria of three closely related trichostrongyle worms is listed in *Table 1*. Our results showed that n=12 in the Suspected Group observed cervical papillae, bursal flap with spicule and vulval flap. These worms presented a dark red colour when collected but the colour was paler when taken out from the 10% formalin.

Also, it is important to note that *H. contortus* is also known as “Barber’s pole worm” due to the blood-filled intestines twisted with the ovaries of the females that give an appearance of the red-white striped pole in barber shops. This feature was not clearly visible to the unaided eyes in all our samples. However, certain other identifying features were visible under 10x and 20x magnification (marked as ▲ in *Table 1*). This

strengthens our suspicion of the worms being *H. contortus*. These highly suspected worms were selected and used for PCR assay confirmation.

PCR Result

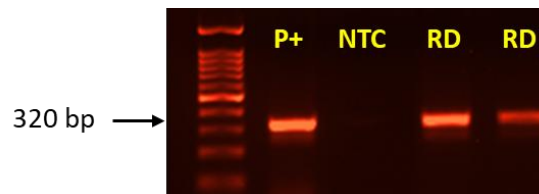
The result of the ITS2 species-specific PCR of the suspected worms is presented in *Figure 2*. The agarose gel electrophoresis resulted in a clear band at the expected 320bp location (*Figure 2*). This confirmed that those worms that satisfied the above key identification points were indeed *H. contortus* parasites. The PCR of the parasite specimen from the Unknown Group showed a negative result (data not shown).

Table 1: Morphological identification keys of three common trichostrongyle worms (Taylor et al., 2015)

	<i>Haemonchus contortus</i>	<i>Trichostrongylus axei</i>	<i>Ostertagia</i> spp.
Gross appearance	<ul style="list-style-type: none"> • Large worms, • Reddish when fresh[▲] • bursa visible with the naked eye 	<ul style="list-style-type: none"> • Very small worms • Greyish when fresh 	<ul style="list-style-type: none"> • Slender • Reddish-brown when fresh
Head/Anterior End	<ul style="list-style-type: none"> • Distinct cervical papillae[▲] 	<ul style="list-style-type: none"> • No cervical papillae 	<ul style="list-style-type: none"> • Cervical papillae more posteriorly present • Cuticle striations
Female	<ul style="list-style-type: none"> • In sheep, usually linguiform vulval flap[▲] • In cattle, rod-shaped vulval flap 	<ul style="list-style-type: none"> • No vulval flap 	<ul style="list-style-type: none"> • Small or absent vulval flap
Male	<ul style="list-style-type: none"> • Dorsal ray of the bursa is asymmetric[▲] • Spicules with a barbed end[▲] 	<ul style="list-style-type: none"> • Symmetrical bursal lobes • Spicules unequal in length 	<ul style="list-style-type: none"> • Asymmetrical bursa • Varied spicules depending on host species
Length (mm)	<ul style="list-style-type: none"> • 10–20 (male) • 18–30 (female) 	<ul style="list-style-type: none"> • 3–6 (male) • 4–8 (female) 	<ul style="list-style-type: none"> • 7–8 (male) • 9–12 (female)

▲: features observed in our specimen in the Suspected Group

Figure 2: ITS2 species-specific PCR result of the suspected worms on agarose gel electrophoresis. 100bp ladder. P+: Positive control DNA; NTC: Non-template control DNA; RD: DNA from the suspected worms



It should be noted here that the roe deers were reportedly “farmed” deer and the hunters reported that it was common for the deer to share the grazing areas with domesticated sheep and cattle. As such, the deer were not dewormed regularly unlike the sheep flocks. Although *H. contortus*, is globally considered the single most important pathogenic parasite in the sheep and goat industry, the parasite has been suggested that it could be transmitted from wildlife hosts to sheep (Halvarsson et al., 2022; Nilsson, 1971). Moreover, results from various wildlife studies with experimental conditions and simulations have reported the capability of the cross-transmission of the parasite between sheep/cattle and other wildlife species including alpine ibex, white-tailed deer, red deer and roe deer (Chintoan-Uta et al., 2014; Halvarsson et al., 2022; Laca Megyesi et al., 2020; McGhee et al., 1981). A more recent study (Beaumelle et al., 2022) reported that the majority of the GIN species found in the roe deer were those commonly found in small ruminant livestock such as *H. contortus*, whereas the more specialised wild cervid nematode species like *O. leptospicularis* were only present at low frequencies. The result of our case study presented here also agrees with the above-mentioned findings, which were also previously reported in Hungary (Csivincsik et al., 2017; Nagy et al., 2017).

CONCLUSIONS

With rising pressure on forested areas and competition for pasture land access, the frequent

sharing of wild and domestic ruminants on common pastures is becoming a concern. This also raises the question of the role of wild species in the transmission of GIN fauna between domesticated and wild animals. The rise of anthelmintic resistance also compounded the detrimental effect of this as there is already an apprehension of reduction of the ‘refugia’ population of GIN parasites in the pastures, which is important to reduce drug resistance. The similar feeding habits of wild and domestic small ruminants could be potentially a higher risk for the transmission of GIN from the wild to the domestic side, yet the reverse cannot be ruled out either. More research into this aspect is necessary to have a better understanding of the parasite dynamics.

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