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Routine microscopy examination of faecal samples as a tool for detection of common gastrointestinal parasites: a preliminary report from two Hungarian farms

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SUMMARY

Gastrointestinal parasitism in ruminant animals is a cause of major economic loss incurred by the livestock industry. Regardless of the frequency of the adopted therapeutic and prophylactic deworming strategies, the parasitic burden in a farm should be assessed regularly. One of the most widely used techniques to do so is the microscopic faecal egg examination and faecal egg counting method. Despite the technique being almost a century old from its first adoption, the principle behind the newer techniques of faecal egg examination is the same. This technique is still being used in routine farm screening and monitoring gastrointestinal parasitic load and faecal egg count reduction testing to assess the anthelmintic efficacy of the drugs used. Thus, the tool remains a choice for preliminary screening for important parasites and the subsequent deworming strategy. Our study here was part of a larger survey on the treatment efficiency as well as a broad epidemiological study of the trichostrongyle parasites in Hungary. We present a preliminary report on the detection of common gastrointestinal parasites from two farms in Hungary, including a species-specific confirmatory microscopy for Haemonchus contortus eggs.

Keywords: *Trichostrongyle egg; faecal egg count; microscopy technique; small ruminants; Haemonchus contortus*

INTRODUCTION

Gastrointestinal (GI) parasitism of small ruminants generally occurs as mixed infections and trichostrongyle nematodes are accepted as the most pathogenic and economically important parasites of small ruminants also (Besier et al., 2016b; Qamar and Maqbool, 2012). These parasites of ruminants could adversely affect their hosts either clinically or economically. The main key factors of nematodiasis can be broadly studied as: i) parasite factors (including the epidemiology of causative parasite), ii) host factors (genetic, physiological, immunological status of the host) and iii) environmental factors (climate, nutrition, stocking density and managemental system) and these factors play an important role in the management of the disease too (Tariq et al., 2008). Thus, depending on these factors, clinical forms of nematodiasis are manifested as signs and symptoms in the dermal, gastrointestinal, or cardiovascular systems. On the other hand, the economic losses manifest in the form of lesser than the genetic potential rate of feed conversion ratio, reproduction capability, or degraded production of milk or meat (Craig, 2018).

Parasitic gastroenteritis is commonly defined as the disease complex of nematodes affecting ruminant animals. The genera most often associated with this complex are *Haemonchus*, *Ostertagia*, *Teladorsagia*, and *Trichostrongylus* (in the abomasum); *Trichostrongylus*, *Cooperia*, and *Nematodirus* (small intestine); and *Oesophagostomum* in the abomasum, small intestine and the large intestine respectively. The eggs of these genera are thin-shelled and segmented, but cannot be readily differentiated except for *Nematodirus* spp., which has a distinctive large oval-shaped egg (Besier et al., 2016a; Craig, 2018; Soulsby, 1968; Taylor et al., 2015). This class of eggs are commonly referred to as trichostrongyle-type eggs and they can be easily differentiated from whipworm or tapeworm eggs in the microscopic examination, but difficult among themselves.

Despite this difficulty in differentiation within the trichostrongyle worm family, the principle and application of faecal egg counting remain important. Various flotation techniques to recover and process the faecal egg count (FEC) of the parasite eggs from faecal samples are available today (Nielsen, 2021) with their characteristic sensitivity and specificity. A classic technique using an egg counting chamber is the McMaster method (Gordon & Whitlock, 1939), which is still widely respected as a laboratory standard to date although newer techniques such as FECPAK (Presland et al., 2005), Mini-FLOTAC® (Cringoli et al., 2017) and Kubic FLOTAC Microscope system (Cringoli et al., 2021) are also available.

Keeping in view the importance of haemonchosis but its relative difficulty in differentiation under conventional microscopy, a species-specific technique using peanut agglutinin (PNA) and fluorescein isothiocyanate (FITC) is also available that specifically detects *H. contortus* eggs under appropriate wavelength filter (Abbas and Hildreth, 2019; Jurasek et al., 2010). Presented here are preliminary results of faecal egg counting and examination, including a confirmatory diagnosis of *H. contortus*, of two farms in south-eastern Hungary. The study was a component of a broader study that aimed to investigate the occurrence of GI parasites of the trichostrongyle family and the effect of drugs administered.

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MATERIALS AND METHODS

Sampling and animals ethics

The faecal samples used were collected from the animals as per the Hungarian Animal Protection and Welfare Act (Act XXVIII of 1998, 3.§). The faecal samples of Farm TZ used were part of a larger study (Toth, unpublished) that was done to screen the faecal egg count of common trichostrongyle worms in Hungary. These faecal samples were collected par-rectally from randomly selected individual animals (n>20) as per the guidelines given in (Coles et al., 1992) with slight modifications. Whereas, the faecal samples from Farm GF were submitted as pooled faecal samples reportedly collected from the shed/ground by the farmer personally. Both the farmers reported suspicion of parasitic infection despite regular deworming.

Faecal egg examination under the microscope

FEC was performed using the Mini-FLOTAC® along with the Fill-FLOTAC® system with saturated salt solution (specific gravity of 1.200; dilution ratio of 1:10) as the floatation medium following exactly the protocols described by Cringoli et al. (2017) for small ruminants. Additionally, to confirm the presence of *H. contortus*, a species-specific microscopy technique known as PNA-FITC fluorescence microscopy, as described by Jurasek et al. (2010), was done using an Olympus BX51 microscope and fluorescence filter (480–490 excitation/527/30 emission). All photographic records were taken using a DP70 camera (Olympus) mounted on an Olympus BX51 microscope and the cellSense software (Olympus).

RESULTS AND DISCUSSION

Faecal egg count, parasitic load and parasite eggs

The result of FEC of trichostrongyle worms for the samples from Farm TZ and Farm GF is given in *Table 1*. Both the farms were found to be positive for the presence of various nematode parasites and some coccidial species also (data not shown as it is irrelevant for this study). Farm TZ (n>20) reported a higher FEC average of 385.8 egg per gram (epg) faeces, with n=4 individual animals showing more than 250 epg of trichostrongyle worm eggs, with suspected *H. contortus* eggs. These suspected individual faecal samples were further analysed with PNA-FITC fluorescence microscopy. It is worth noting that the parasitic burden of Farm TZ can be reported as a 'light infection' as given by Taylor et al. (2015). This low intensity of infection could be attributed to the fact that the animals of Farm TZ were regularly dewormed. Moreover, the degree of clinical manifestation of parasitic gastroenteritis complex in general, and haemonchosis in particular, should always be assessed with other clinical signs and symptoms, body condition, deworming history as well as with other species-specific confirmatory diagnoses. The same was also true for Farm GT (mean FEC of 189.37 EPG) although the deworming history of the farm is unknown/not reported by the farmer.

Table 1: **Mean FEC of the farms**

	Trichostrongyle nematodes (egg per gram)	Remarks
Farm GF	189.3	Pooled faecal samples from a goat farm
Farm TZ	*385.8Ω	*n=4 individual sheep had *H. contortus* suspected eggs

Ω: individual animal FEC data is not shown as it will be used for another report (unpublished)

The eggs of the GI parasites observed in this preliminary report can be seen in *Figure 1*. As mentioned earlier, only the *Nematodirus* spp eggs (*Figure 1-A*) can be easily identified by their relatively large size and oval-shaped eggshell with a clearly visible embryo inside. Other species of parasites visible in the microscopic examination were Strongyloides worm which could be seen as larvated eggs (*Figure 1-B*); tapeworm eggs which could be suspected of *Moniezia* spp (*Figure 1-B*) by their distinctive triangular-shaped egg with thick walls and the faintly visible pyriform apparatus inside; suspected coccidial type ova (*Figure 1-C*); *H. contortus* suspected eggs (*Figure 1-C*) and confirmed *H. contortus* egg (*Figure 1-D*) by PNA-fluorescence microscopy. For large sample sizes, the composite method of FEC using pooled sampling also provides significant advantages in time-saving without compromising on the result accuracy (Nicholls and Obendorf, 1994). It should be noted that certain artefacts (as seen in *Figure 1-C*) could also be present. They could be pollen grains, undigested feed particles and air bubbles. Outmost care was taken so that the artefacts were not confused/misdiagnosed as parasitic eggs.

Figure 2 presents a panel of images, adapted from Taylor et al. (2015), of commonly observed eggs of various helminth parasites of ruminant animals, including those of sheep and goats. It can be inferred from this that the eggs of the trichostrongyle-type (middle panel, *Figure 2*) are quite similar and can be easy to be misdiagnosed when it comes to the species level. This is important for a proper targeted/selective deworming strategy, such as in the case of haemonchosis management. Our study here also reported the main advantage of PNA-FITC microscopy in accurately diagnosing the *H. contortus* egg from the samples of the suspected animals. Under PNA-FITC fluorescence microscopy, the eggs of *H. contortus* gave a distinctly clear outline stained in green colour against a dark background (*Figure 1-D*). This is characteristic of the eggs of this parasite species only (Abbas and Hildreth, 2019) which is otherwise hard to distinguish from other similar trichostrongyle-type eggs observed under normal microscopy (*Figure 1-C; Figure 2*).

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Figure 1: **Microscopy photographs of various parasite species of small ruminants**
A: *Nematodirus* spp. 10x magnification; B: Mixed infection with suspected tapeworm (TW) and unidentified strongyloides worm (ST). 20x magnification; C: Mixed infection with suspected *H. contortus* (H^) and coccidial species (CX). 20x magnification; D: Confirmed *H. contortus* (H++) under fluorescence microscopy. 20x magnification

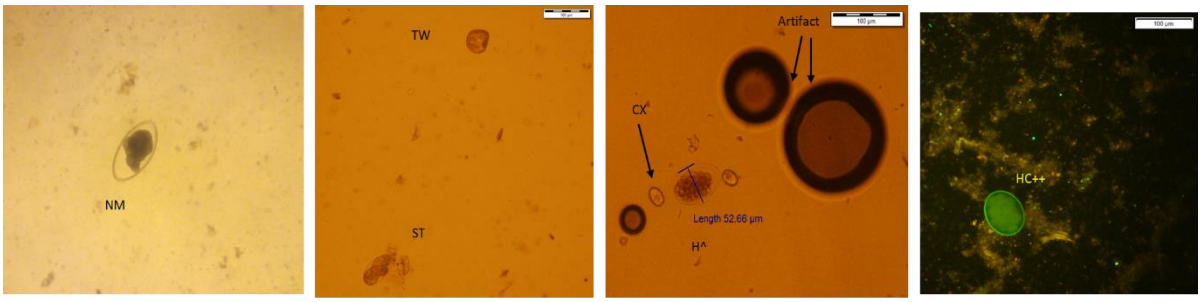
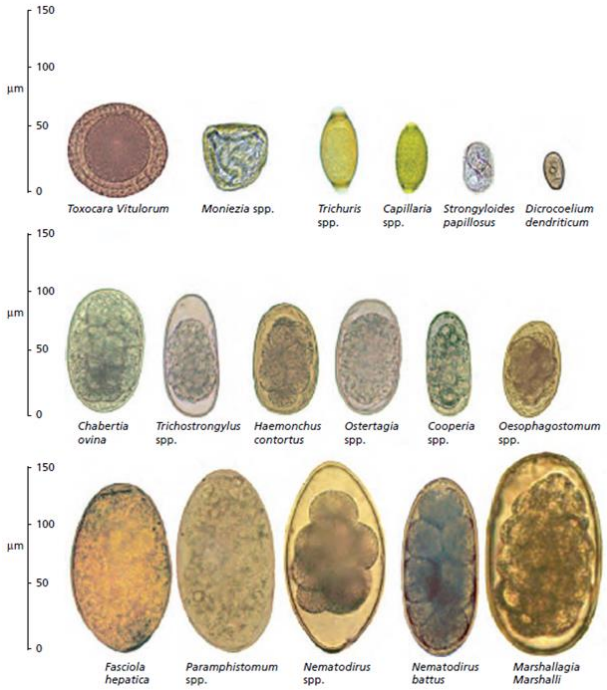


Figure 2: **Parasitic eggs of common helminths of ruminants**



Note: Image adapted from the book "Veterinary Parasitology" by Taylor, M.A.; Coop, R.L and Wall, R.L

CONCLUSIONS

Although it has been over 100 years since the first parasite faecal egg counting techniques were designed, faecal egg counting and identification remain valid and a requirement in parasitology research as well as routine clinical examination even to this day. The technique has been refined from time to time and more sophisticated and even automated tools are being developed to reduce the cost, time and expertise dependency. Yet, despite some of its demerits, the conventional faecal egg count and examination prove an indispensable tool. Our preliminary report also aligned with this and the data obtained from this study can be used to contribute to a general screening tool to detect the presence of the important gastrointestinal nematode parasites in Hungary, and if a more detailed and species-specific diagnosis is required or not depending on the worm epidemiology.

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