DOI 10.2478/s11687-009-0012-0

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HELMINTHOLOGIA, 46, 1: 62 – 64, 2009

## Research Note

# Use of modified McMaster method for the diagnosis of intestinal helminth infections and estimating parasitic egg load in human faecal samples in non-endemic areas

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**Summary**

A modified McMaster method has been used for the diagnosis and estimating helminth egg load in human faecal samples obtained from random consecutive patients in the areas non-endemic for helminth infections (Slovak Republic, North West Russia). Both positive and negative findings were in a 100 % concordance to those obtained with a reference method accepted in clinical diagnostic laboratories (microscopy of the native stool smear). The McMaster method was efficient in detecting nematode eggs in patients' stool samples with egg load varying from very low (15 – 60 epg for *T. trichiura*) to moderate (1650 – 4500 epg for *A. lumbricoides*). Therefore, this method may be successfully (and with a better technical feasibility) used for the diagnosis of intestinal helminth infections in non-endemic areas, with further quantitative analysis of the sample when required.

Key words: McMaster method; helminth eggs; human faecal samples; diagnosis; non-endemic area

**Introduction**

The McMaster technique developed at the McMaster laboratory of the University of Sydney is the most commonly used technique for identifying and quantitation of parasite elements in faeces. Its use has been described in every detail with many variations in the evaluation of faecal egg counts in small ruminants, horses, cows, pigs, also in the species being studied less often, such as alpacas and llamas (Cebra, Stang, 2008), rabbits (Rinaldi *et al.*, 2007), turtles (Giannetto *et al.*, 2007), mice (Meyer-Lucht, Sommer, 2005), lemurs (Schad *et al.*, 2004), camels (Bekele, 2002), and even red grouses (Seivwright *et al.*, 2004). In humans, McMaster technique has been employed only for quantitative estimation of the effect of anthelminthic drugs in endemic areas: mebendazole against hookworms (Flohr *et al.*, 2007), albendazole against hookworms, *Trichuris* and *Ascaris* (Stephenson *et al.*, 1989), mebendazole against *Trichuris* (Sargent et al., 1975), pyrantel and piperazine against *Ascaris* (Bell, Nassif, 1971), and tetramizole against *Ascaris*, hookworms and *Enterobius* (Thienpont *et al.*, 1969).

In all aforementioned studies, the McMaster technique was used in the areas with a high prevalence of parasitic infections and high parasite and egg loads per patient. However, in order to most completely evaluate the diagnostic sensitivity of any clinical laboratory test, it has to be applied in cohorts with both high and low prevalence of a disease under study. The reason for it is such that calculation of the test sensitivity presumes the inclusion of both diagnosed (true positive) and missed (false negative) cases of the disease, which greatly vary between endemic and non-endemic areas (Marshall, 2000). Additionally, a clinical laboratory assay has to be tested for analytical stability, which, again, should involve the evaluation of the test in the cohorts with high and low prevalence of the disease being diagnosed (Groth, 1990). Nonetheless, nothing has been known about the sensitivity of McMaster method in non-endemic areas with no connection to anthelminthic therapy of already recognised helminthic infections.

**Materials and methods**

The study was performed on stool samples (single sample per patient) obtained at the admission of patients to the Second Clinic for Children and Adolescents (Košice, Slovak Republic) and to an out-patient centre (polyclinic of the North West Research Centre for Hygiene and Public Health, St. Petersburg, Russia). The samples were taken for parasitological examination when indicated by a referring clinician. For the purposes of quality assessment, all samples were examined independently by two highly qualified technicians using a native stool smear, and the examination findings were kept blind until completing the

McMaster assay.
The latter was performed according to the guidelines of the World Association for the Advancement of Veterinary Parasitology (Coles *et al.*, 1992). Briefly, the test employed a two-chambered, engraved slide (both chambers were of 0.5 ml volume), onto which a supernatant of the faecal suspension in saturated sucrose was placed. The saturated sucrose solution instead of sodium chloride employed in the original technique was chosen because of a better recovery of *Trichuris* (Cebra, Stang, 2008) and *Strongyloides* (Crignoli et al., 2004) eggs in the former solution.
The flotation time in the chambers was not less than 30 minutes, which standardised the recovery of helminth eggs (Dunn, Keymer, 1986). Another way of standardising the technique and improving its precision was calculating all the eggs in both chambers instead of one (Coles *et al.*, 1992).

**Results and discussion**

Out of 32 consecutive patients (male: 18, female: 14, age: 11 – 47 years) examined with the modified McMaster method for the period of May – July 2008 at both clinical settings, 8 patients infected with intestinal helminths were detected (Table 1). Only *Ascaris lumbricoides* and *Trichuris trichiura* eggs were detected at both laboratories. In all cases, both positive and negative findings were in a 100 % concordance to those obtained on faecal smears (no false-positive or false-negative results have been identified). In turn, there was a 100 % agreement between the findings by the two technicians having assayed faecal smears independently of each other.

Table 1. Comparison of the findings obtained with the McMaster technique and qualitative (reference) assay in patients infected with *Ascaris lumbricoides* and/or *Trichuris trichiura*

| Positive cases | Stool smear tests | | | | Egg count per gram faeces | |
|---|---|---|---|---|---|---|
| | Technician 1 | | Technician 2 | | | |
| | *Ascaris lumbricoides* | *Trichuris trichura* | *Ascaris lumbricoides* | *Trichuris trichura* | *Ascaris lumbricoides* | *Trichuris trichiura* |
| #1 | Positive | Positive | Positive | Positive | 4500 | 15 |
| #2 | Negative | Positive | Negative | Positive | - | 60 |
| #3 | Positive | Negative | Positive | Negative | 2775 | - |
| #4 | Positive | Positive | Positive | Positive | 3600 | 150 |
| #5 | Positive | Negative | Positive | Negative | 1650 | - |
| #6 | Negative | Positive | Negative | Positive | - | 240 |
| #7 | Negative | Positive | Negative | Positive | - | 45 |
| #8 | Positive | Negative | Positive | Negative | 4065 | - |

The results have shown that McMaster technique may be successfully used for the diagnosis of intestinal helminth infections in a non-endemic area, with egg load varying from very low (15 - 60 epg for *T. trichiura*) to moderate (1650 – 4500 epg for *A. lumbricoides*). Its diagnostic performance was equal to that of a reference assay (native stool smear), despite the patient cohort has been small. Detecting and identifying helminth eggs with McMaster method is much easier technically than with the native stool smear, and better images can be obtained on photomicrographs of the eggs for further study when necessary. Quantitative assessment of the sample provided by the McMaster method is an extra benefit for a clinician willing to quantitatively evaluate the efficacy of a specific antiparasitic therapy if required.

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RECEIVED AUGUST 25, 2008

ACCEPTED JANUARY 22, 2009