

Enteric parasites in high-prevalence settings – a challenge for the diagnostic routine ?

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Objectives. Facultative pathogenicity is a challenge for diagnostic microbiology. Positive test results do not necessarily mean associations with symptomatic disease if pathogens are detected in primarily non-sterile compartments. Harboring billions of microorganisms, stool is such a compartment. In this study, we analyzed the occurrence of infestations with enteric parasites in stool samples of apparently healthy school children in rural Madagascar. Positive test results were correlated with typical symptoms of enteric disease.

Methods. In a population of 388 apparently healthy Madagascan school children, stool samples were collected and analyzed by multiplex real-time PCR for enteric protozoa (*Entamoeba histolytica*, *Giardia duodenalis*, *Cryptosporidium* spp., *Cyclospora* spp.) and helminths (*Ascaris lumbricoides*, *Ancylostoma* spp., *Necator americanus*, *Strongyloides stercoralis*) after DNA extraction using the Qiagen® stool kit as described by the manufacturer. In addition, stool material was microscopically analyzed after filtration and sedimentation for eggs of enteric helminths according to Madagascan diagnostic standard procedures. For *Giardia duodenalis*, *Cyclospora* spp., and *Ascaris lumbricoides*, for which relevant numbers of infestations were observed, prevalence ratios for the symptoms 'vomiting', 'diarrhoea', 'abdominal pain', 'fever', 'bloody stools', 'fatigue', 'loss of appetite', and 'jaundice' during the previous two weeks were assessed. Positive test results were correlated with typical symptoms of enteric disease.

Results. Altogether, we detected infestations with *Giardia duodenalis* in 195/388 children (50.3%), with *Ascaris lumbricoides* in 72/388 (18.6%), with *Cyclospora* spp. in 62/388 (16.0%), and with *Strongyloides stercoralis* and *Cryptosporum* spp. in 1/388 (0.3%) each, respectively. DNA or eggs of hookworms were not observed. The prevalence ratios (+/- confidence intervals) suggested few relevant associations of detections of *Giardia duodenalis*, *Cyclospora* spp., and *Ascaris lumbricoides* with the analyzed symptoms. Diarrhoea and bloody stool were less frequent in case of infestations with *Ascaris lumbricoides*. In contrast, vomiting, abdominal pain, and loss of appetite were seen more frequently in case of infestations with *Cyclospora* spp., and loss of appetite more frequently in case of infestations with *Giardia duodenalis*.



	<i>Ascaris</i> microscopy or PCR positive	95% CI	<i>Cyclospora</i> spp. PCR positive	95% CI	<i>G. duodenalis</i> PCR positive	95% CI
	72/388		62/388		195/388	
vomiting	0.74	0.48-1.13	1.43	1.02-2.00	1.06	0.79-1.41
diarrhoea	0.58	0.36-0.94	1.34	0.96-1.87	0.90	0.68-1.20
abdominal pain	1.00	0.79-1.28	1.28	1.03-1.59	0.80	0.66-0.97
fever	0.93	0.71-1.20	0.89	0.67-1.18	0.90	0.75-1.10
bloody diarrhoea	0.53	0.31-0.90	1.07	0.71-1.62	0.83	0.60-1.13
fatigue	0.80	0.56-1.15	1.17	0.86-1.59	0.81	0.63-1.03
loss of appetite	0.90	0.63-1.29	1.43	1.07-1.92	1.45	1.11-1.90
jaundice	0.33	0.10-1.02	1.38	0.70-2.73	0.38	0.20-0.72

Table: Prevalence ratios for symptoms depending on different test results. Reported symptoms during the last 2 weeks before the investigation.

Conclusions. As known from experimental treatment of inflammatory bowel disease with *Trichuris suis*-associated immune modulation, infestations with helminths were associated with less enteric symptoms. However, even the effects of *Cyclospora* spp. on enteric symptoms were relatively mild and relevant effects of *Giardia duodenalis* were nearly not observed, suggesting some kind of semi-immunity or tolerance.

Accordingly, the interpretation of positive test results for enteric parasites in patients with infectious gastrointestinal disease requires the consideration of high rates of apathogenic infestations in high-prevalence settings.

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