SHORT ARTICLE

Microscopic inter-observer reliability of intestinal parasitic infections in trained laboratory technicians of rural Mexico

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Abstract

Intestinal parasitic infections caused by *Giardia lamblia* (GL), *Ascaris lumbriocoides* (AL) and *Entamoeba histolytica/dispar* (Eh/Ed) are highly prevalent among indigenous groups in Mexico. In resource-constrained settings, direct microscopic fecal examination continues to be a common diagnostic method in spite its limited accuracy. This study aimed at illustrating the effect of training local laboratory technicians from a rural reference hospital located in a marginalized indigenous region of northern Mexico to assess the inter-observer reliability of GL, AL, and Eh/Ed diagnoses. Two experienced technicians working at the hospital were trained and standardized for two full weeks in the Parasitology Laboratory at the National Children's Hospital from Mexico City. Diagnoses were made by microscopy of two serial stool samples processed using the modified Faust zinc sulphate centrifugal flotation technique to concentrate AL eggs and GL and Eh/Ed cysts. Observations were done independently, and the final diagnosis for each observer was established when at least one of the two samples resulted positive. Reliability analyses from serial stool samples were conducted using Cohen's kappa correlation coefficient (κ) for each parasite. Agreement between observers reached 88.7, 72.4, and 80.5% for Eh/Ed, AL, and GL, respectively. Largest kappa coefficient was observed for GL (κ=0.55), followed by Eh/Ed (κ=0.30), and AL (κ=0.08). Prevalence of Eh/Ed, AL and GL according to observers 1 and 2 were 3.4 vs. 13.5%, 4.0 vs. 28.2%, and 32.2 vs. 33.3%, respectively. Except for GL, reliability was very low leading to major differences in prevalence estimates. These results question the value of training technicians, as intestinal parasitic microscopic diagnoses seemed to be very difficult to replicate between observers questioning their validity, leading to differences in clinical decisions, and in prevalence estimates.

Key Words

Amoeba; ascaris; giardia; intestinal parasites; inter-observer reliability; microscopy; training

Introduction

Intestinal parasitic infections caused by *Giardia lamblia* (GL), *Ascaris lumbriocoides* (AL) and *Entamoeba histolytica/dispar* (Eh/Ed) are the second leading cause of morbidity and mortality in Mexico (1), and are especially prevalent in poor indigenous populations (2,3).

They persist in remote areas with poor hygiene and high environmental risk factors (4). In Mexico, Eh/Ed has the highest incidence followed by AL and GL with 288, 53 and 13 cases per 100,000 in 2013,

respectively (5). However, data should be seen carefully, as diagnostic methods used are mostly clinical or based on microscopic fecal exam, which limit accuracy significantly (6,7).

From various techniques available to identify these parasites, direct microscopy of stool samples is widely used in resource-constrained settings (7,8) due to its relative accessibility and low cost (9). Despite its limited accuracy (10-15), studies suggest that microscopic exam can be a cost-effective tool to detect intestinal parasites such as GL and AL (9,16) provided that diagnoses are made after rigorous training of laboratory technicians (6,16).

However, there is insufficient data on the value of training individuals in terms of reliability to diagnose specific parasites. In fact, we were unable to find any published studies looking at the inter-observer reliability between laboratory technicians after training to identify the three parasites studied here using microscopy. While producing the same diagnosis does not imply valid results, failure to do limit the chance of accurate diagnoses.

Aims & Objectives

To illustrate the effect of training local technicians working in the laboratory of a rural reference hospital located in a marginalized indigenous region of northern Mexico to assess the inter-observer reliability for GL, AL and Eh/Ed diagnoses.

Material and Methods

We performed reliability analyses between two laboratory technicians for the identification of GL, AL and Eh/Ed using serial stool samples. This was a part of a school-based trial to prevent intestinal parasitic infections among indigenous children using an intervention that included anti-parasitic treatment, modifications in school infrastructure, and implementation of educational measures (17).

Children from two indigenous boarding schools, located in a poor, marginalized, and mountainous region of northern Mexico were screened for intestinal parasites. Children attend school during weekdays where they receive food, shelter, and education, and return back for the weekends to their homes usually located in small, scattered and isolated settlements with cold weather in the hills and subtropical in the gorges. From the 222 children registered at both schools, 196 were present during the baseline visit. Adequate samples were available for 194 and 184 children for AL/GL and Eh/Ed analyses, respectively.

Diagnoses were made by microscopy of two consecutive stool samples. Fecal specimens of 15-20 g were placed in clean plastic containers and preserved in 10% formaldehyde solution for up to three days before microscopic examination at the laboratory of the largest reference hospital in the region.

Samples were processed using the modified Faust zinc sulphate centrifugal flotation technique to concentrate AL eggs, and GL and Eh/Ed cysts (18). Microscopic observations were made by the two experienced technicians working at the hospital, trained and standardized for two weeks in the Parasitology Laboratory at the National Pediatric Hospital of Mexico City. The diagnosis of each parasite was established by the two technicians independently when at least one of the two samples was positive.

The proportion of agreement and Cohen's kappa correlation coefficient were computed to assess the inter-observer reliability. Data was analyzed using SPSS version 22.

This original study was approved by the National Council for Science and Technology (FOMIX CONACyT-Chihuahua grant No. 23223) and by the National Commission for the Health of Indigenous Peoples. Parents or tutors provided informed consent, and children themselves gave witnessed verbal consent. Individualized nitazoxanide treatment was given to children with positive diagnoses.

Results

For Eh/Ed, AL and GL, agreement between observers reached 88.7, 72.4, and 80.5%, respectively. The largest kappa coefficient was observed for GL (κ=0.55), followed by Eh/Ed (κ=0.30), and AL (κ=0.08). Prevalence of Eh/Ed, AL and GL according to observers 1 and 2 was 3.4 vs. 13.5%, 4.0 vs. 28.2%, and 32.2 vs. 33.3%, respectively. (Table 1)

Discussion

Accurate diagnoses of parasitic infections must rely on methods with high sensitivity and specificity to be of value for public health purposes (19). However, when such methods are subjected to human error, training becomes crucial to produce valid results (20). In the case of certain parasitic infections, not only is the use of serial samples essential when using direct microscopy (16), the expertise of the laboratory technician also becomes necessary to improve the probability of correct diagnoses (20).

While it is true that there are better methods to diagnose intestinal parasitic infections, it is also a fact that the use of microscopy continues to be widely used in many middle- and most low-income settings, including Mexico and India, in spite of its low accuracy and reliability that leads to poor diagnoses and inadequate treatment.

We measured the reliability of diagnoses for three common intestinal parasites between two local technicians trained in a major laboratory of a national hospital. Except for GL, kappa coefficients were very low, especially for AL, leading to major differences in prevalence estimates between observers. While good reliability does not assure diagnostic validity, poor reliability does seriously question it. Based on these findings, we can only recommend the use of better methods to limit misdiagnoses.

Small laboratories located in remote locations require better infrastructure and resources to be able to run tests that are subjected to less human error. While molecular methods to diagnose parasitic species is currently one that requires expensive infrastructure and highly trained personnel (21, 22), efforts should be made to incorporate the use of immunoenzymatic techniques to improve the diagnoses of these and other parasites (23, 24).

In conclusion, our results question the value of training technicians, as intestinal parasitic microscopic diagnoses seemed to be very difficult to replicate between observers questioning their validity, leading to differences in clinical decisions, and in prevalence estimates. We believe that our paper sends a clear public health message to health authorities, as it questions the use of microscopic examination of stool samples to diagnose intestinal parasites, and thus raises awareness of the need to use more reliable and accurate tools.

Conclusion

Small laboratories located in remote locations require better infrastructure and resources to be able to run tests that are subjected to less human error. While training can improve microscopic diagnoses, the use of better methods (e.g. immune enzymatic) is required to obtain more reliable results.

Recommendation

Based on these findings, we can only recommend the use of better methods to limit misdiagnoses.

Authors Contribution

All authors contributed equally.

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Tables

TABLE 1 RELIABILITY BETWEEN TWO LABORATORY TECHNICIANS USING DIRECT MICROSCOPY TO DIAGNOSE *ENTAMOEBA HISTOLYTICA/DISPAR*, *ASCARIS LUMBRICOIDES*, AND *GIARDIA LAMBLIA* FROM TWO SERIAL STOOL SAMPLES AMONG INDIGENOUS SCHOOLCHILDREN OF NORTHERN MEXICO

Trained laboratory technician		Observer 2, n (%)		Total	Cohen's kappa coefficient
		Positive	Negative		
Entamoeba histolytica/dispar					
Observer 1, n (%)	Positive	5 (2.8)	1 (0.6)	6 (3.4)	0.30
	Negative	19 (10.6)	153 (85.9)	172 (96.6)	
	Total	24 (13.5)	154 (86.5)	178	
Ascaris lumbricoides					
Observer 1, n (%)	Positive	4 (2.3)	3 (1.7)	7 (4.0)	0.08
	Negative	45 (25.9)	122 (70.1)	167 (96.0)	
	Total	49 (28.2)	125 (71.8)	174	
Giardia lamblia					
Observer 1, n (%)	Positive	40 (23.0)	16 (9.2)	56 (32.2)	0.55
	Negative	18 (10.3)	100 (57.5)	118 (67.8)	
	Total	58 (33.3)	116 (66.7)	174	