doi: 10.5216/rpt.v48i1.57760

ORIGINAL ARTICLE

EVALUATION OF CLINICAL LABORATORIES IN DIAGNOSING INTESTINAL PARASITIC INFECTIONS

Regielly Caroline Raimundo Cognialli[1,2] and Débora do Rocio Klisiowicz[1,3]

ABSTRACT

Parasitic infections are an important public health issue due to their high prevalence and widespread incidence. In Brazil there are no data on the performance of clinical laboratories regarding fecal examinations. The purpose of this study was, therefore, to assess the performance of clinical laboratories in Curitiba and its Metropolitan Region, Paraná state, on the diagnosis of intestinal parasitic infections. Samples were sent to laboratories in three semi-permanent preparations on glass slides for microscopy analysis and three samples in diluent solution. The forwarded samples contained ten different parasite species and 22 possible diagnoses. The laboratories were scored very good, good, average or below average according to a predetermined standard. None of the assessed laboratories scored very good regarding the diagnosis of intestinal parasitic infections, 21.1% of laboratories scored good, 15.8% average and 63.2% below average. There were 22% false positives and 24.4% false negative results. The diagnosis of *Ascaris lumbricoides* eggs was least mistaken. The most common diagnostic failures were in the identification of hookworm larvae, *Iodamoeba bütschlli* cysts and *Fasciola hepatica* eggs. The poor performance of laboratories in parasitological diagnosis demonstrated that parasitology laboratories are neglected and professional training is not up to standard.

KEY WORDS: Clinical laboratories; performance; intestinal parasites.

INTRODUCTION

Parasitic infections are an important public health problem due to their high prevalence and widespread incidence (Biolchini, 2005; Abraham et al., 2007; Pisarski, 2019). According to the World Health Organization (WHO) over 3.5 billion people are infected by intestinal parasites, 450 million of which are ill, mostly children (UNICEF, 1998; Okyay et al., 2004; WHO, 2013).

1. Post Graduation Program in Microbiology, Parasitology, Basic Pathology Departament, Federal University of Parana, Curitiba, Brazil.
2. Clinicas Hospital Complex, Federal University of Parana, Curitiba, Brazil.
3. Basic Pathology Department, Federal University of Parana, Curitiba, Brazil.

Corresponding author: Regielly Caroline Raimundo Cognialli, Complexo Hospital de Clínicas, Universidade Federal do Paraná, Rua Padre Camargo, 280, CEP 80060-240, Curitiba, Paraná, Brazil. E-mail: regielly.cognialli@gmail.com

Received for publication: 19/3/2019. Reviewed: 8/5/2019. Accepted: 9/5/2019.

Adequate diagnosis in fecal examination is essential, as the therapeutic procedure is different for each type of parasite (Rana & Misra-Bhattacharya, 2013; Mrus et al., 2018, Palmeirim et al., 2018).

Scientific studies on diagnostic quality control in parasitology laboratories are rare. Most of the studies are performed in hematology, biochemistry and microbiology laboratories (Sardiñas et al., 2016; Mao et al., 2018; Souza et al., 2018). The few studies published on parasitology laboratories are mainly from Latin America (Ayala & Sánchez, 1974; Castro et al., 1995, Laird et al., 1997, Núñez et al., 1998; Núñez & Finlay, 2001; Blanco et al., 2013; Jemere et al., 2018).

There are no published data on the performance of parasitology laboratories in Brazil. The present study aims to evaluate the performance of clinical analysis laboratories in Curitiba and its Metropolitan Region in the State of Paraná on the diagnosis of intestinal parasitic infections.

MATERIAL AND METHODS

In order to carry out assays, ten different parasites species were selected. The stage form and the type of sample (microscope slide or suspension) in which the parasites were delivered to the laboratories studied are described in Table 1. Each clinical laboratory received three semi-permanent preparations on glass slides for microscopy analysis, specified as "microscope slide", and three samples in 1 mL of preservative solution (10% formaldehyde) specified as "suspension". For suspension analysis the laboratories were instructed to prepare a microscope slide with a drop of the suspension with Lugol's iodine and analyze it under the microscope. Before sending the samples to the participating laboratories all preparation settings were evaluated by two professionals with over two years experience in fecal examinations in order to ascertain the presence of all the parasites in the samples.

The study was approved by the Research Ethics Committee of the Federal University of Paraná, under registration CAAE: 11331512.2.0000.0102. Over 32 clinical laboratories in Curitiba and its Metropolitan Region were contacted by telephone to participate in the research project.

The criteria for inclusion in the study were acceptance of free participation in the study and signature of the Free and Clarified Consent Term. Laboratories that did not carry out parasitological examination of feces or did not agree to sign the Free and Clarified Consent Term were excluded from the study.

Table 1. Parasite species, stage form and type of samples delivered for analyses in the study.

Preparation	Parasite and stage form
Microscope slide 1	*Ascaris lumbricoides* eggs
	Trichuris trichiura eggs
	Enterobius vermicularis eggs
	Hymenolepis nana eggs
	Taenia sp. eggs
	Hookworm eggs
	Giardia duodenalis cysts
	Entamoeba coli cysts
Microscope slide 2	Hookworm rhabditiform larvae
Microscope slide 3	*Ascaris lumbricoides* eggs
	Trichuris trichiura eggs
	Enterobius vermicularis eggs
	Giardia duodenalis cysts
	Entamoeba coli cysts
	Iodamoeba bütschlli cysts
Suspension 1	*Ascaris suum** eggs
	Taenia sp. eggs
	Giardia duodenalis cysts
Suspension 2	*Fasciola hepatica* eggs
	Candida albicans yeast
Suspension 3	*Fasciola hepatica* eggs
	Taenia sp. eggs
	Giardia duodenalis cysts
	Candida albicans yeast

**Ascaris suum* eggs are morphologically identical to *Ascaris lumbricoides*.

The clinical laboratories were graded according to a system stipulated for this assay, each correct identification corresponding to two points, and each error to one point. According to the number of correct or wrong parasite identifications, the participating laboratories were graded very good (90-100%), good (80-89%), average (70-79%) and below average (<69%). At the end of the project, the participating laboratories received a report with their performance in the study.

RESULTS

Only nineteen out of the 32 laboratories invited to take part in the research project accepted, ten private and nine public; therefore approximately 24% of the laboratories in the region were investigated. The 13 laboratories that refused stated they had no interest in participating.

All the participating laboratories informed that the exams were analyzed by a professional with higher education, in 17 laboratories by pharmacists, one by a biomedical scientist and one by a biologist.

The number of correct diagnoses, scores, false-positive and false-negative results analyzed are shown in Table 2. 58% of the laboratories reported a larger number of parasites and all laboratories made incomplete diagnoses. An average of 22% false-positive and 24.1% false-negative results were observed. According to the grading system the laboratories were classified as good (13.3%), average (50%) and below average (26.7%).

Table 2. Number of correct diagnoses, scores, false-positive, false-negative and final classification regarding the performance of the assessed laboratories

Laboratory code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Number of correct diagnoses	21	19	19	11	13	21	20	20	19	14	20	15	20	21	12	12	13	14	12
Score	36	28	31	9	12	36	30	36	29	13	32	21	31	39	4	12	15	16	8
False-positive	5	7	4	2	5	5	8	2	6	7	6	2	7	2	10	2	2	4	6
False-negative	1	3	3	11	9	1	2	2	3	8	2	7	2	1	10	10	9	8	10
Classification	G	I	R	I	I	G	I	G	I	I	R	I	R	G	I	I	I	I	I

Note: VG – Very good; G – Good; R – regular; I – Insufficient

Table 3 shows the percentage of parasites correctly identified in each type of preparation. Regarding helminths, *Ascaris lumbricoides* eggs were correctly identified on both slides by all laboratories, but only 90% identified them in suspension (number 1). *Hymenolepis nana* eggs were sent on slide number 1 and 94% of the laboratories correctly identified this parasite. *Enterobius vermicularis* eggs were sent on two slides (number 1 and 3) and were correctly identified by 84% and 47% of the laboratories respectively. *Trichuris trichiura* eggs were sent on two slides (number 1 and 3) and were identified by 74% and 63% of the laboratories. *Taenia* sp. eggs were sent on microscope slide number 1 and in two suspensions (number 1 and 3) and were correctly identified in 79%, 68% and 63% respectively. Hookworm eggs were sent on one slide (number 1) and identified by 95% of the laboratories. *Fasciola hepatica* eggs were sent in suspensions number 2 and 3 and the identification percentages were 58% and 53% respectively.

Table 3. Percentage of parasites correctly identified by laboratories in each type of preparation

Preparation	Microscope slide			Suspension		
	nº1	nº 2	nº 3	nº 1	nº 2	nº 3
Parasites and stage form delivered	Correct identification of parasites in %					
Ascaris lumbricoides eggs	100	-	100	90	-	-
Hymenolepis nana eggs	94	-	-	-	-	-
Enterobius vermicularis eggs	84	-	47	-	-	-
Trichuris trichiura eggs	74	-	63	-	-	-
Taenia sp. eggs	79	-	-	68	-	63
Hookworm eggs	95	-	-	-	-	-
Fasciola hepatica eggs	-	-	-	-	58	53
Giardia duodenalis cysts	95	-	89	74	-	74
Iodamoeba bütschlli cysts	-	-	37	-	-	-
Entamoeba coli cysts	84	-	95	-	-	-
Hookworm rhabditiform larvae	-	47	-	-	-	-

Regarding protozoa, *Giardia duodenalis* cysts were sent in four samples and in both types of preparation, and were correctly identified in 95% and 89% of the microscope slides and 74% of the suspensions. *Iodamoeba bütschlli* cysts were present on slide number 3 and were identified by 37% of the participating laboratories. *Entamoeba coli* cysts were sent on two microscope slides (number 1 and 3) and were identified by 84% and 95% of the laboratories respectively. Hookworm rhabditiform larvae were sent on microscope slide number 2, but only 47% of the laboratories correctly identified this parasite.

Table 4 shows the false-positive results reported by the laboratories in each type of preparation. Sixteen different parasites that had not been delivered in any type of preparation were reported. Among the false-positive results, it is noteworthy that 58% of the laboratories reported *Endolimax nana* cysts in four samples and 42% of the laboratories mistakenly identified hookworm rhabditiform larvae as *Strongyloides stercoralis* larvae.

Table 4. Percentage of parasites mistakenly identified by laboratories in each type of preparation

Preparation	Microscope slide			Suspension		
	1	2	3	1	2	3
Ascaris lumbricoides eggs	-	-	-	-	11	5
*Hymenolepis diminuta** eggs	11	-	-	-	-	-
Hymenolepis nana eggs	-	-	11	5	-	-
Enterobius vermicularis eggs	-	-	-	-	-	5
Trichuris trichiura eggs	-	-	-	-	-	5
Taenia sp. eggs	-	-	16	-	-	-
Hookworm eggs	-	-	16	26	5	21
*Diphyllobothrium latum** eggs	-	-	-	21	11	11
Entamoeba coli cysts	-	-	-	-	21	11
*Entamoeba histolytica** cysts	5	-	21	-	-	-
Endolimax nana cysts	58	-	58	-	26	21
Giardia duodenalis cysts	-	-	-	-	5	-
*Chilomastix mesnili** cysts	-	-	-	-	5	5
*Balantidium coli** cysts	-	-	-	-	5	-
*Cryptosporidium parvum** oocysts	-	-	-	-	-	5
Strongyloides stercoralis larvae	-	42	-	-	5	-

* Parasites not sent in any preparation
False-positive results in %

DISCUSSION

It is believed that 13 laboratories did not agree to participate in the study because they knew they would be evaluated.

Castro et al. (1995) assessed the health centers in the cities of Lima and Callao, both in Peru, and 26.8% of the laboratories diagnosed more parasites than delivered and 21.8% sent incomplete diagnoses. These results are lower than those observed in the present study in which 58% of the laboratories reported a higher number of parasites and all of them provided incomplete diagnoses. Blanco et al. (2013) reported 58.7% incomplete diagnoses in Bolívar, Venezuela.

The number of false-positive results found in this study (22%) was significantly higher than the results found in a similar analysis in Cali, Colombia (5.8%) (Ayala & Sánchez, 1974). The authors ascribed those results to the awareness of the Colombian population regarding the high prevalence of enteroparasites. Thus, laboratory technicians and other professionals are compelled to find parasites even if these are not evident (Ayala & Sánchez, 1974). In this study the laboratories were aware that they were being evaluated which might explain the false positives percentages.

False-negative results corresponded to 24.4%, less than those observed in Colombia (53.21%) (Ayala & Sánchez, 1974). This result can be due to ignorance of the parasitic forms by the laboratory technicians or the presence of more than one parasite in the same sample. Furthermore, most of the errors related to intestinal parasite diagnosis may be due to the difficulty in identifying protozoa and in detecting eggs and cysts in cases of polyparasitism (Smith, 1979; Eamsobhana & Boranintra, 1989; Hawthorne et al., 1992; Eamsobhana & Boranintra, 1993; Kettelhut et al., 2003).

Ascaris lumbricoides species presented fewer errors with 15.8% false-positive results due to the typical egg morphology and the high parasite load present in the samples.

Ascaris suum decorticated eggs were mistakenly identified by 47% of the laboratories who instead reported the presence of hookworm eggs or *Diphyllobothrium latum* eggs. The possible diagnosis alteration from *Ascaris suum* decorticated eggs to hookworm eggs is worrying as it may indicate lack of knowledge regarding morphology, leading to erroneous epidemiological data.

Enterobius vermicularis eggs were not identified by 53% of the laboratories, and there were 5% false-positive results. The low percentage of accuracy in the diagnosis of this parasite may be because the microscope slide contained only one or two parasite eggs, possibly indicating the microscope slide was not fully examined. However, there is a certain difficulty in recognizing its morphology, since it is not a parasite regularly diagnosed in fecal examinations (Cook, 1994).

Even in samples with a low parasite load, *Trichuris trichiura* eggs present characteristic morphology, however, 47% of the assessed laboratories failed to identify these helminthic eggs on the microscope slides, possibly indicating the microscope slide was not completely examined.

Forty-two percent of the laboratories did not identify *Taenia* sp. eggs on the microscope slides or in suspension, and 16% could not identify the eggs either on the microscope slides or in suspension. This result is quite alarming as an infection triggered by *Taenia solium* may lead to autoinfection causing a cysticercosis condition, of which there is an average worldwide mortality rate of 50.000 individuals per annum (Cruz et al., 1989; Chapman et al., 1995; Eddi et al., 2003).

The microscope slide with hookworms rhabditoid larvae were correctly identified by 47% of the laboratories, 42% diagnosed them as *Strongyloides stercoralis* larvae and 11% reported a negative result. This error may be because in most cases the larval form found in feces is *Strongyloides stercoralis* shaped. This mistake may lead to erroneous treatment. For hookworm infections albendazole, mebendazole, pyrantel pamoate and levamisole treatment are suggested. On the other hand, for strongyloidiasis the drug of choice is ivermectin, but thiabendazole and albendazole can be an alternative treatment (Bethony et al., 2006; Olsen et al., 2009; Khieu et al., 2013; Krolewiechi & Nutman, 2019).

Fasciola hepatica eggs, although operculated and measuring 130-150 µm, were identified only by 53% of the laboratories. This diagnosis difficulty is reportedly common in similar assays (Laird et al., 1997; Núñez et al., 1997; Núñez & Finlay, 2001; WHO, 2004; Sánchez et al., 2012). Such diagnosis failure is probably due to the lack of parasite feature knowledge by the professionals.

The large number of erroneous results directly affects population health and a false-positive result may increase the indiscriminate use of antiparasitic drugs which is a concerning issue as drug resistance may develop during therapy. On the other hand, a false negative result may cause damage to the patients' health, who may remain untreated and act as a disseminator causing persistence of the parasite biological cycle.

According to the predetermined grading, none of the laboratories scored very good in the diagnosis of intestinal parasites, 21% scored good, 16% average and 63% were below average. There was no difference between public and private laboratories. A similar analysis conducted by Castro et al. (1995) but using different criteria stated that 10% of the assessed laboratories ranked very good, 13.3% good, 50% average and 26.7% below average and Blanco et al. (2013) reported 57.1% below average. In the current assessment, the laboratories performed better in the microscope slide analyses than in suspensions, this result is very concerning, since suspensions would only require a little simple manipulation for the preparation of a microscope

slide. Routine materials for fecal examinations are feces *in natura*, therefore requiring more handling, which may result in a higher percentage of errors.

The professionals who perform the fecal examinations apparently tend to have some difficulty in recognizing parasite morphology, since many mistakes could have been avoided if the professional had paid more attention to the size of the cysts, eggs and larvae, as well as peculiarities in the form of the parasitic elements.

The fact that no laboratory scored very good in fecal diagnosis and most of them were below average is quite alarming since it reflects neglect on the part of the laboratories regarding parasitology.

The first step to improve diagnoses is to provide continuous theoretical and practical training for professionals and improve parasitology teaching in the medical courses which should emphasize the importance of parasitological diagnoses.

ACKNOWLEDGEMENTS

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). We thank the clinical laboratories that agreed to participate in this study, CAPES who provided Regielly Caroline Raimundo Cognialli's scholarship and the Microbiology, Parasitology and Pathology graduate Program of the Universidade Federal do Paraná.

REFERENCES

1. Abraham RS, Tashima NT, Silva MA. Prevalênica de enteroparasitoses em reeducandos da Penitenciária Maurício Henrique Guimarães Pereira de Presidente Venceslau - SP. *Rev Bras Anal Clin 39*: 39-42, 2007.
2. Ayala S, Sánchez CE. Evaluacion de los diagnosticos coproparasitologicos realizados en los laboratorios clinicos de la ciudad de Cali. *Acta Méd Valle 5*: 114-121, 1974.
3. Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, Hotez PJ. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet 367*: 1521-1532, 2006.
4. Biolchini CL. Enteroparasitoses na infância e adolescência. *Adolesc Saúde 2*: 29-32, 2005.
5. Blanco Y, Hernández M, Monroy F, Amaya I, Romero M, Rodolfo D. Control de calidad en el diagnóstico coproparasitológico em laboratorios clínicos públicos de Ciudad Bolívar, Venezuela. *Saber 25:* 166-175, 2013.
6. Castro J, Yovera J, Núñez F. Control de calidad del diagnostico coproparasitologico en centros de salud de Lima y Callao. *Rev Peru Epidemiol 8*: 18-22,1995.
7. Chapman A, Vallejo V, Mossie KG, Ortiz D, Agabian N, Flisser A. Isolation and characterization of species-specific DNA probes from *Taenia solium* and *Taenia saginata* and their use in an egg detection assay. *J Clin Microbiol 33*: 1283-1288, 1995.
8. Cook GC. *Enterobius vermicularis* infection. *Gut 35*: 1159-1162, 1994.

9. Cruz M, Davis A, Dixon H, Pawlowski ZS, Proano J. Operational studies on the control of *Taenia solium* taeniasis/cysticercosis in Ecuador. *Bull World Health Organ 67*: 401-407, 1989.
10. Eamsobhana P, Boranintra K. Identification of fecal parasites in the quality assessment programme for the year 1984-1987, in Thailand. *J Med Assoc Thai 72*: 11-15, 1989.
11. Eamsobhana P, Boranintra K. Parasitology proficiency testing in the quality assessment programme in Thailand. *J Med Assoc Thai 73*: 626-630, 1993.
12. Eddi C, Nari A, Amanfu W. *Taenia solium* cysticercosis/taeniosis: potential linkage with FAO activities; FAO support possibilities. *Acta Trop 87*: 145-148, 2003.
13. Hawthorne M, Chiodini PL, Snell JJ, Moody AH, Ramsay A. Parasitology: United Kingdom National Quality Assessment Scheme. *J Clin Pathol 45*: 968-674, 1992.
14. Jemere KA, Melaku MY, Jemeber TH, Abate MA. Performance evaluation of laboratory professionals on malaria microscopy at health facilities in Bahir Dar city administration, Northwest Ethiopia. *PloS One 13*: 2-10, 2018.
15. Kettelhut MM, Chiodini PL, Edwards H, Moody A. External quality assessment schemes raise standards: evidence from the UKNEQAS parasitology subschemes. J *Clin Pathol 56*: 927-932, 2003.
16. Khieu V, Schar F, Marti H, Sayasone S, Duong S, Muth, S, Odermatt P. Diagnosis, treatment and risk factors of *Strongyloides stercoralis* in schoolchildren in Cambodia. *PLoS Negl Trop Dis 7:* 1-8, 2013.
17. Krolewiecki A, Nutman TB. Strongyloidiasis: A Neglected Tropical Disease. *Infec Dis Clin N Am 33:* 135-151, 2019.
18. Laird PR, De Risco BU, Ramírez FE, Gallardo DJ, González GC, Crespo AF. Estudio de la calidad del diagnostico coproparasitologico en dos provincias de Cuba. *Kasmera 25*: 155-169, 1997.
19. Mao X, Shao J, Zhang B, Wang Y. Evaluating analytical quality in clinical biochemistry laboratory using Six Sigma. *Biochem Med 28*: 1-4, 2018.
20. Mrus J, Baeten B, Engelen M, Silber SA. Efficacy of a single-dose 500 mg mebendazole in soil-transmitted helminth infections: a review. *J Helm 92*: 269-278, 2018.
21. Núñez FA, Ginorio DE, Finlay CM. Control de la calidad del diagnóstico coproparasitológico en la provincia de Ciudad de La Habana, Cuba. *Cad Saúde Publ 13*: 67-72, 1997.
22. Núñez FA, Ginorio DE, Cordocí RA, Finlay CM. Intervención educativa para mejorar la calidad del diagnóstico coproparasitológico en la red de salud de Ciudad Habana, Cuba. *Cad Saúde Publ 14:* 139-144, 1998.
23. Núñez FA, Finlay CM. Adiestramiento en el diagnóstico de las parasitosis intestinales en la red de laboratorios de Cuba. *Cad Saúde Publ 17*: 719-724, 2001.
24. Okyay P, Ertug S, Gultekin B, Onen O, Beser E. Intestinal parasites prevalence and related factors in school children, a western city sample- Turkey. *BMC Public Health 4*: 1-6, 2004.
25. Olsen A, van Lieshout L, Marti H, Polderman T, Polman K, Steinmann P, Stothard R, Thybo S, Verweij JJ, Magnussen P. Strongyloidiasis--the most neglected of the neglected tropical diseases? *Trans R Soc Trop Med Hyg 103*: 967-972, 2009.
26. Palmeirim M, Hürlimann E, Knopp S, Speich B, Belizario Jr. V, Joseph SA, Michel Vaillant M, Olliaro P, Keiser J. Efficacy and safety of co-administered ivermectin plus albendazole for treating soil-transmitted helminths: A systematic review, meta-analysis and individual patient data analysis. *Plos Neglec Trop Dis 12*: 1-26, 2018.
27. Pisarski K. The Global Burden of Disease of Zoonotic Parasitic Diseases: Top 5 Contenders for Priority Consideration. *Trop Med Infec Dis 4*: 1-9, 2019.
28. Rana AK, Misra-Bhattacharya S. Current drug targets for helminthic diseases. *Parasitol Res 112:* 1819-1831, 2013.

29. Sánchez RM, Cañete ID, Marcelo JCM, Santos AP. Fascioliases, clinical- epidemiological review and diagnosis. *Rev Cubana Hig Epidemiol 50:* 88-96, 2012.

30. Sardiñas M, Garcia G, Martínez MR, Díaz R, Mederos LM. Importancia del control de la calidad de la baciloscopia en los laboratorios de diagnóstico de tuberculosis. *Rev Chil Infec 33*: 282-286, 2016.

31. Smith JW. Identification of fecal parasites in the special parasitology survey of the College of American Pathologists. *Am J Clin Pathol 72*: 371-373, 1979.

32. Souza AKN, Cordeiro PGM, Souza CL, Oliveira MV. Review of peripheral blood smear slides: assessment of the compliance with criteria used by analysts in a laboratory of a public hospital in Bahia, Brazil. *J Bras Patol Med Lab 54*: 220-226, 2018.

33. UNICEF (Fundo das Nações Unidas para a Infância). *Situação Mundial da Infância*. Brasília, 1998. 248p.

34. World Health Organization. *Training manual on diagnosis of intestinal parasites*. Geneva, 2004. 130p.

35. World Health Organization. *Parasitic Diseases*. Geneva, Switzerland. 2013. 210p.