International Scholars Journals

African Journal of Parasitology Research ISSN: 2343-6549 Vol. 4 (5), pp. 219-226, May, 2017. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Comparative study between the Baermann conventional and a simplified Baermann devices for the diagnosis of *Strongyloides stercoralis*

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Accepted 18 May, 2017

The-conventional Baermann device components are a constraint during its use for field investigations. A simplified version is proposed in order to assess the agreement of the measurements and the cost between both devices. 101 stool samples from HIV positive subjects were examined using both devices. The positivity rate was 0.9-% (1/101). The stool tested positive was subjected to 7 successive dilutions by means of a *Strongyloides stercoralis* larvae free stool. Each dilution, considered as a sample, was used to compare the accuracy agreement of both devices. The Student t test did not show any significant variation in the number of the extracted larvae per gram of stool (p=0.227). The correlation coefficient (0.9991) close to 1 indicates a high reliability between both devices. The measurement of the larvae (per gram of stool) using both devices agree as shown by the Bland Altman analysis, with a positive bias. Bias = 0.5 ± 1.069; 95% CI (- 0.39 to 2.59). The cost of the analysis was estimated at $ US 30.31 for the conventional device versus $US 2.23 for the simplified one. The latter is an alternative for the strongyloidiasis diagnosis during large-scale investigations in countries with limited resources.

Keywords: Strongyloidiasis, diagnosis, Baermann, devices, Comparison, agreement, Abidjan, Côte d'Ivoire.

INTRODUCTION

The World Health Organisation (WHO) ranks Strongyloidiasis fifth worldwide among the Soil-transmitted helminth (STH) infections. This is an endemic infection in tropical and subtropical regions of the globe. 30 to 100 million people worldwide suffer from it (Bethony J et al., 2006; Hotez PJ et al., 2006; Olsen A et al., 2009). It affects mostly farmers (Ngui R et al., 2016) and subjects living in rural areas but also, subjects who frequently walk bare foot in the mud in the urban and sub-urban areas where sanitation infrastructure is inadequate. Generally, it is asymptomatic in 20 to 40 -% of cases (Valerio L et al., 2013). In the symptomatic cases, the clinical manifestations are intestinal, pulmonary and cutaneous. Immunosuppressed subjects tend to develop a-hyperinfection syndrome and disseminated strongyloidiasis threatening the life of the patient-(Buonfrate D et al., 2013; Yılmaz l et al., 2013; El-Sameed YA et al., 2015; Nabeya D et al., 2017). Among the stool analysis method implemented for the biological diagnosis of the disease, the direct microscopic examination can help diagnose by discovering

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Strongyloides stercoralis rhabditoide larvae-in the fresh stool. However, the laying of larvae by the females is often minimal and their emission is irregular in the stool (Schar F et al., 2013; Wegayehu Y et al., 2013). This examination fails to detect the larvae results-(Agrawal V et al., 2008) mainly due to its low sensitivity (Ericsson CD et al., 2001). The conventional Baermann technique-was set up in 1917 (Baermann G-and Eine einfache, 1917). It is a concentration technique which has a better sensitivity (Garcia LS, 2001). Other methods were proposed and which could increase the detection of S. *stercoralis* larvae. They are: the culture on Harada-Mori filter paper, agar plate culture and, PCR (Machicado et al., 2012; Ghasemikhah R et al., 2017; Wang LF et al., 2017).

These techniques are the most expensive though. The conventionnal Baermann technique becomes therefore-a required key element to obtain reliable data on the strongyloidiasis prevalence. (Garcia LS, 2001). However, most studies reporting on the incidence of the disease did not use it, making the prevalence of this disease often underestimated compared to other STH infections species (Menan EIH et al., 1997; Bethony J et al., 2006; Hotez PJ et al; 2006, Olsen A et al., 2009). In Côte d'Ivoire, there are only a few data on epidemiological studies of strongyloidiasis available. Previous studies using the Baermann method report prevalence rates ranging from 2.7% to 48% (Djohan V et al., 2010, Glinz D et al., 2010). The conventional Baermann technique requires a great number of glass funnels, and their transport and cleaning constraints are an obstacle to its use in the field investigations (Hernández-Chavarría FandAvendaño L, 2001). Nevertheless, this method is carried out in laboratories to detect *S. stercoralis* larvae in stool (Ketzis JK, 2017). It had already been subjected to modifications in order to make it simpler (Hernandez-Chavarria F, 2001; Graeff-Teixeira C, 1997; Ketzis JK, 2017).

In this work, we are presenting a simplified version of the conventional Baermann device. It was used to detect *S. stercoralis* larvae in large-scale investigations in Côte d'Ivoire. In the framework of this study, this simplified device is compared to the conventional one which is the method of reference.

METHODOLOGY

Study areas and Tested stool

This is an experimental study for the diagnosis of Strongyloidiasis. It was carried out from August 2009 until October 2010 in the parasitology and mycology laboratory of Aids and opportunistic diseases Research and Diagnosis Center (CeDRes) in Abidjan (Ivory Coast).

The stool samples were collected from HIV positive hospitalized or consulting out patients in the pneumophtisiology (PPH) and the infectious and tropical diseases departments of the University medical centers (CHU) of cocody and Treichville respectively.

Presentation of the Baermann devices

The conventional Baermann device consists of a glass funnel fixed on a wooden stand. This funnel is prolonged by a rubber tube clamped with a Morh claw (Photo-1).

The simplified Baermann device was made with a 300 ml capacity plastic bottle of mineral water sold in stores. The top third of the plastic bottle which was removed using a pair of scissors, and closed with the cup of the bottle, was used in the reverse position as a funnel. This funnel is adapted to the lower two third of the bottle used as support (Photo 2).

METHODS

The Baermann method was carried out using both devices. Only one examination was done with each device. The procedure used is the following: we put a small square piece of gauze in a metal sieve, and then a layer of 'Kleenex' paper tissue on which we deposited 10 g of stool. The corners of the gauze were folded on top and all of it was deposited on the glass or plastic funnel which was closed. The funnel was filled with twenty ml of warm water (30 – 45 °C). In order to extract the larvae, the bottom of the sieve barely touches the surface of the water. When present, the larvae leave the stool to concentrate in the water. After 1 to 3 hours, the extraction water was collected in a centrifuge tube. After centrifugation for 3 to 5 minutes at 3000 RPM the sediment was collected and examined by light microscopy. The larvae were spotted thanks to their mobility after magnification X 10. In the case of positive examination the quantification of *S. stercoralis* larvae was given per gram of stool. This quantification was done as follows: N represents the number of larvae contained in the 10 g of stool used to carry out the Baermann methods. The extraction liquid was supposed to contain all the larvae present in the tested stool. The number of larvae per gram was estimated using a proportionality rule. In both cases, the determination of the parasitic load was done by the same microscopist.

In the case of a high density of larvae in the stool (impossibility to count the larvae), a series of successive dilutions of the tested positive stool was undertaken. The dilutions were done using fresh stool tested negative for *S. stercoralis* larvae. In the case of low density the dilution was not necessary. Each dilution was considered as a sample. The sample was measured twice: once with the conventional device and a second time with the simplified device. The costs of the equipment used in both devices were also estimated and compared.

Statistical analysis

Many tests and methods were used, notably the Student t test, the correlation coefficient method (r) used for

Photo 1. Conventional Baermann device.

Photo 2. Simplified Baermann device.

correlation analysis. Linear regression test between the devices was also performed. The Bland Altman analysis (Bland J Mand Altman DG, 1986), used to compare both devices. The significance degree of the tests is fixed at level α = 5-%. P values < 0.05 were considered significant. The soft-wares-Excel 2007, SPSS and XL Stat. were used.

RESULTS

101 stool samples, including 63 from male and 38 from female patients, 34 to 66 years old (37 years ±3. 2) were collected. The search for *S. stercoralis* larvae was positive for only one patient, which represents a positivity rate of 0.9%. It was a 34-year-old male subject, suffering from malignant strongyloidiasis. The subject was infected by HIV1, his rate of LT4 was 182 cells per mm^3, and he belonged to the C category of the CDC classification. The direct examination of the fresh stool samples showed very mobile *S. stercoralis* larvae. The larvae density was high (147 larvae/gram of stool). Seven successive dilutions were done (1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128). The larvae density per gram corresponding to each dilution is shown in table 1. The Student t test showed no significant variation in the number of larvae

Table 1. Measurement of larvae density for each dilution: conventional versus simplified Baermann devices.

Dilutions	Number of larvae / g of stool		P-value
	Conventional device	simplified device	
1/2	21	22	
1/4	16	16	
1/8	11	10	
1/16	9	8	0.227
1/32	6	7	
1/64	5	6	
1/128	3	4	
Total	71	73	

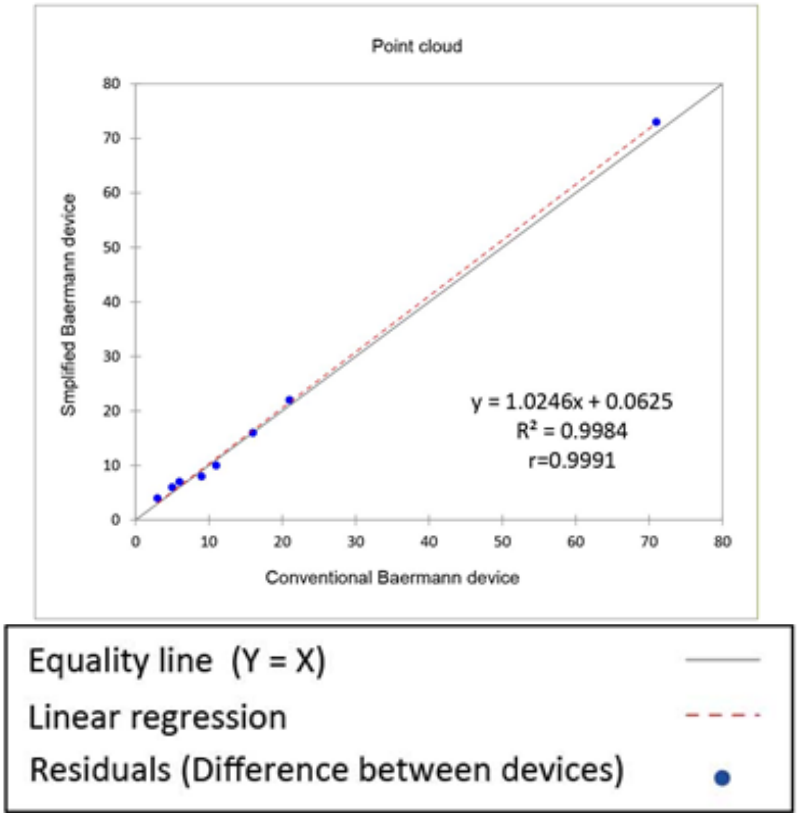

Figure 1. Correlation between the measurements obtained by conventional and simplified Baermann devices (linear regression).

extracted per gram of stool for both devices (p=0,227). The regression line related to the measurement from both Baermann devices is close to the bisector (line of equality y=x) but above this one. The correlation coefficient (r) is estimated at 0.9991 and proves that there is an extremely close link between the variables (Figure 1). The study of the conformity of both Baermann devices using the Bland-Altman analysis showed a positive bias (Figure 2): Bias = 0.5 ± 1.069; 95% CI (-0.39 to2.59).

The cost of the analysis is estimated at US $ 30.31 for the Baermann conventional device against US $ 2.23 for the proposed new simplified device. The list and the cost of the input components of each device are shown in table 2.

DISCUSSION

The strongyloidiasis prevalence rate that we are reporting (0.9%) is lower than the one found in Brazil: (11/63) representing 17% (Graeff-Teixeira C et al, 1997). The difference of the modus operandi used during the study carried out in Brazil could explain our result. In the Brazilian study, the extraction liquid (at room

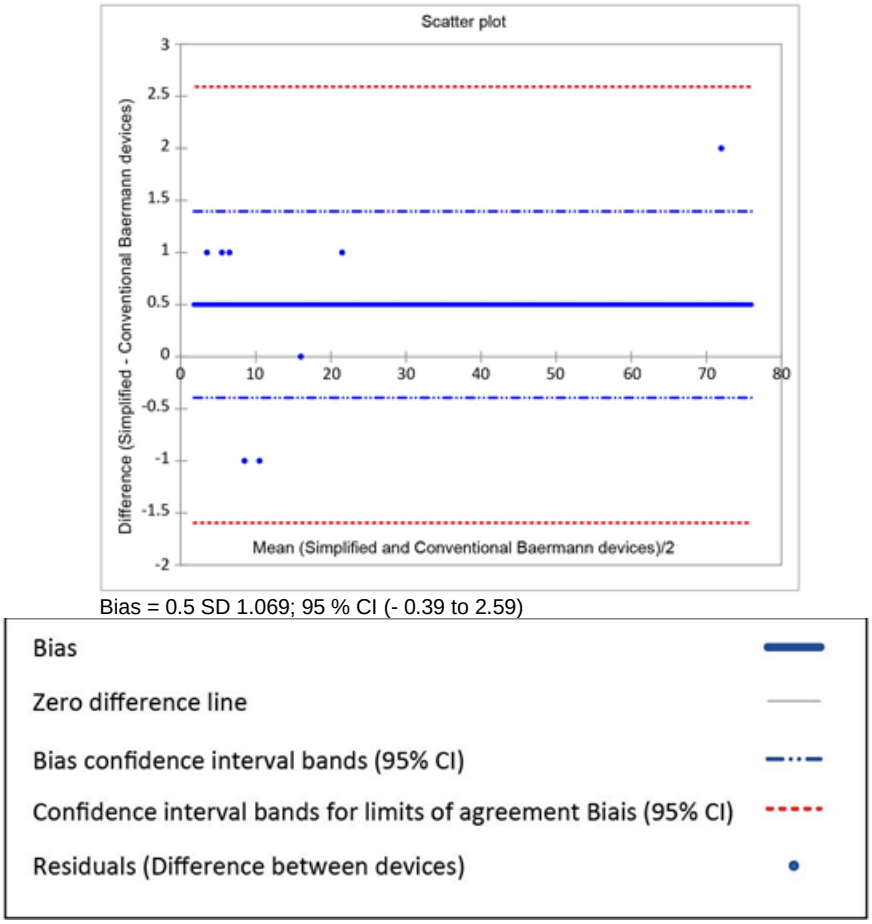

Figure2. Degree of agreement between larvae measurement (Bland Altman plot) by Conventional and simplified Baermann devices.

Table 2. Comparison between the estimated cost in US Dollar of the components used in the conventional and simplified Baermann devices.

Components designation	Components cost in US Dollar	
	Conventional	Simplified
Tongue depressor	0.040	0.040
Object carrying slide	0.097	0.097
Cover glass	0.048	0.048
Gauze	0.161	0.161
Plastic pipette	0.161	0.161
Metal sieve	0.242	0.242
Conical-bottomed centrifuge tube	0.809	0.809
Protective gloves	0.024	0.024
Stand	1.294	-
Mohr claw	4.852	-
Glass funnel	22.647	-
300 ml plastic bottle	-	0.646
Total	30.375	2.228

temperature: 25-30°C) is collected after about 12 hours. The extraction liquid is analysed without centrifugation. This modus operandi could have probably improved the sensitivity of the method. In the same study, the author uses another procedure with the extraction time reduced to 2 hours. He notes a lower sensibility explained by the

small quantity of the stool sample used (1g). We believe that an execution procedure longer than three hours can be proposed in the laboratories which do not have a centrifuge despite the long execution period. This is often the case in first contact health centers in developing countries.

The frequent use of only one stool sample examination by the Baermann technique, sometimes explains the reporting of low *S. stercoralis* infection rates (Kassi FK et al., 2008; Menan EIH et al., 2008;-Djohan V et al., 2010). The repetition of the number of examinations enables a correct diagnosis of the strongyloidiasis. Studies report that the diagnosis increased to 50% with 3 samples of stool and reached 100% if 7 stool samples are examined (Nielsen PB and Mojon M, 1987; Sato Y et al., 1995).

The Strongyloidiasis infection diagnosis is easy. The Baermann extraction method remains the reference method used routinely. The simplified Baerman device that we are proposing, is similar to the one proposed in 1997 to diagnose intestinal strongyloidiasis among patients in Brazil (Graeff-Teixeira C et al., 1997). However there are some differences in the equipment used. In the case of the study conducted in Brazil, the author uses a 2 liter plastic bottle, and the funnel is closed with a small rubber baloon. In our case, the bottle used has a less capacity (300 ml), provides a better stability of the device. Morever using the cap of the botle to close the plastic funnel is easier. However the operator is exposed to a transcutaneous contamination risk by the *S. stercoralis* larvae when collecting the extraction liquid. This risk could be avoided by using protecting gloves.

The various modifications brought to the classical Baermann device (De Carli GA, 1994; Willcox HP and Coura JR, 1989) answer to the problem of having a simple diagnostic stool, of simple use to search for *S. stercoralis* larvae mostly for field investigations.

In this work we compared the agreement between the measurements of both devices. Our work is limited because we used only one stool sample. The use of the student t test for 2 paired samples as a statistical test, lies on the hypothesis that the differences of measurements are normally distributed and evaluates the risk to conclude that both devices are different. This risk is high (22.7%), whereas there is not any difference. The study of the regression line and of the spread the cloud of point obtained during our experimental measurement, does not indicate any error in the number of extracted larvae during the experimentation of both devices. This statistical approach does not provide any information on the statistical value of the bias and on the limits of the confidence interval at 95% in which the measurements are contained. However the correlation coefficient is close to 1 (r=0.9991), proving that there is an extremely close link between both devices. Furthermore, the correlation coefficient does not constitute a conformity indicator, as the correlation between two measurements can be perfect even if the values of one represent the double of those of the other (Bland JM and Altman DG, 1986). Testing the conformity between two series of observations thus implies the use of other statistical method. In front of the inadequacy of the linear regression methods to compare two different devices for identical measurements, a conformity study method lies on the analysis of the biais by a graphic representation (Bland JM and Altman DG, 1986). The determination of the confidence interval at 95% of the biais enables to appreciate if the differences are clinically acceptable (Ennouchi F et al., 2002). The approach used to evaluate the larvae count in this work requires to be further studied. This evaluation is all the more important as it could help to relate the parasitic infection and the symptoms observed during this disease.

Besides the diagnostic aspect, the cost of the analysis is also important in the implementation of the method. In fact, in a study conducted in 15 public Laboratories and 14 private ones to detect *S. Stercoralis* larvae, 5 laboratories use the Baermann technique. One of the main reasons that justify the use of that technique is its cost (Ketzis JK, 2017). The spontaneous sedimentation technique in tube is also effective for the strongyloidiasis diagnosis. It was proposed in Peru for poor heathcare settings and under field-working conditions at about US $0.03 (Tello R et al, 2012).

CONCLUSION

The Student t, the linear regression tests and the Bland Altman analysis enable us to conclude that the conventional and the simplified Baermann devices agree. The modified version of the Baermann device revealed itself to be a good tool for the extraction of *S. stercoralis* larvae. The use of the plastic bottle enabled us to reduce the cost of the analysis. Its reduced cost and the simplicity of its use are in favor of its popularization in parasitology laboratories of countries with limited resources. The use of this tool in large-scale epidemiological investigations enable us to determine the actual *S. stercoralis* infection prevalence underestimated for a long time. This is the starting point of an efficient fight. This preliminary work should be carried on, using a larger sample.

Conflict of interest

The authors declare that they have no conflict of interest

Authors' contribution

Hervé Menan supervised the study. Pulchérie Christiane Marie Kiki-Barro, analysed and drafted the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENT

Thanks to all patients who helped to carry out this work by giving their consent, not forgetting to mention the

CeDres staff that made all arrangements for the examinations to be carried out.

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