Research Article

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Diagnostic performance of the Willis, Ritchie and Bailenger methods in Parasitic Coprology

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Abstract

Intestinal parasitosis is a disease caused by either a protozoan or a helminth, associated with faecal contamination. In Benin, gastrointestinal diseases rank third in prevalence after malaria and acute respiratory diseases, with a prevalence of 6.6%. This study evaluates the diagnostic performance of the Willis, Ritchie, and Bailenger methods in parasitic coprology in the commune of Aguégués in the Ouémé department of Benin. Macroscopic examination of stool samples included assessment of consistency, presence of non-faecal elements, and adult worms. Prior to microscopic examination using the Willis, Ritchie, and Bailenger techniques, stool samples were examined with physiological water. The overall prevalence of parasitism among the 202 participants was 40.10%, with 81 individuals found to be parasitised. Among the parasitised participants, 59.26% were female. *Ascaris lumbricoides* was the most prevalent parasite species identified (56.80%), followed by *Ancylostoma duodenale* (29.22%) and *Balantidium coli* (13.58%). Bailenger's method proved to be the most effective (40.10%), with Ritchie's (38.11%), Willis' (30.19%), and direct examination with physiological water (17.32%) following closely behind. This article challenges the common practice in medical diagnostic laboratories of limiting parasitic coprology to direct examination with physiological water, as the results presented here question its effectiveness.

Keywords: Willis, Ritchie, Bailenger, protozoosis, helminthiasis, Benin

Introduction

Parasitosis is a disease caused by the presence of an organism (parasite) that depends on another, more organised organism (host) that provides it with the elements necessary for its development. It threatens the socio-economic development of developing countries with a very high morbidity rate (Bourée, 2015). The World Health Organization (WHO) estimates that more than 1.5 billion people, or nearly 24% of the world's population, are infested with geohelminths (OMS, 2022). Geohelminth infections are among the most common in the world, affecting the poorest and most disadvantaged communities. Ascariasis, amoebiasis, hookworm and trichocephaliasis are among the ten most common infections worldwide (Zouitni, 2022). Although they are not associated with high mortality, complications are not uncommon and many cases require hospitalisation. The environmental situation in most African countries in the intertropical belt and in underdeveloped countries such as Benin, where the majority of the population lives, is conducive to the development of parasitosis. However, much research has been done on the diagnosis of these diseases. In Benin, several studies in recent years have found low prevalence rates. In addition, few laboratories carry out concentration methods. This observation prompted this study to perform concentration techniques using the Willis, Ritchie and Bailenger methods to confirm or refute previous studies. In the literature, the performance of concentration methods is recommended during parasitic coprology (ANOFEL, 2016). The present work aims to contribute to the fight against digestive parasitosis by evaluating the diagnostic performance of the Willis, Ritchie and Bailenger methods in parasitic coprology for better management of users of medical diagnostic laboratories in Benin.

Materials and Methods

Material

The biological material consisted mainly of fresh stool samples. The laboratory equipment used included a light microscope and a centrifuge. The reagents consisted of acetoacetic buffer, ether, saturated NaCl solution (25 g/L), formalin and 2% Lugol's iodine.

Methods

This was a cross-sectional study carried out in the municipality of Aguégués on 202 stool samples. Socio-demographic data were collected through a questionnaire administered to the participants. The participants were informed of the correct conditions for collecting the samples, which were collected in a clean, dry, wide-mouthed plastic container with a label identifying the participant. For the analytical phase, we performed macroscopic examination (consistency, non-faecal additions), direct examination with physiological water, direct examination after staining with Lugol's, the Willis, Ritchie and Bailenger methods. The aim of the concentration techniques is to eliminate any interfering elements (food particles, etc.) from the stool in order to facilitate the identification of the parasites under the microscope at objective 40, after focusing and searching at magnification 10. In the Willis technique, stool samples are mixed with a saturated solution of sodium chloride (NaCl) with a density greater than that of the parasites. This allows the lighter parasite eggs to float to the surface where they can be collected with a microscope slide. Using Ritchie's physico-chemical method (Azzouz, 2020), 5 g of faeces were diluted with 10 times its volume of formalin solution (10%) to obtain a homogeneous suspension. The mixture was sieved. Ether was added to the filtrate, emulsified and centrifuged at 1500 rpm for 3 minutes. The preparation was examined at objectives 10 and 40. For the Bailenger method (Malenou, 2017), 2 g of stool sample was placed in a 10 ml conical-bottomed tube and 2.5 ml acetoacetic buffer was added. The resulting mixture was allowed to settle and then filtered through a gas compress. We then added 2.5 ml of ether, mixed and centrifuged at 2500 rpm for 10 minutes. The pellet was examined between slide and coverslip at 40x magnification.

Results

Description of the study population

The present study identified 202 volunteer participants who provided stool samples for parasitic coprology.

Sex distribution of the study population

Figure 1 shows the gender distribution of the study population.

Figure 1 : Distribution of study population by gender

The figure above shows that 59.26% of the participants were female.

Age distribution of study population

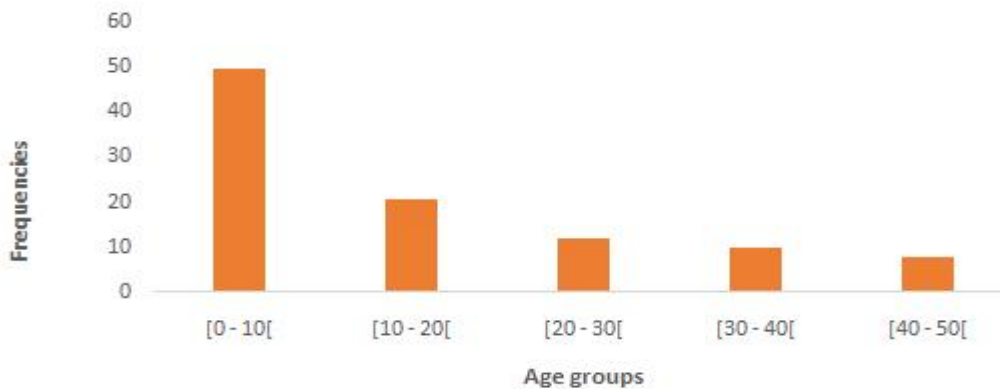The figure below shows the age distribution of the study population.

Figure 2: Breakdown of the study population by age group

Analysis of this figure shows that almost half (49.5%) of the study population is aged between 0 and 10 years.

Distribution of the study population by level of education

Figure 3 shows the distribution of the study population by level of education.
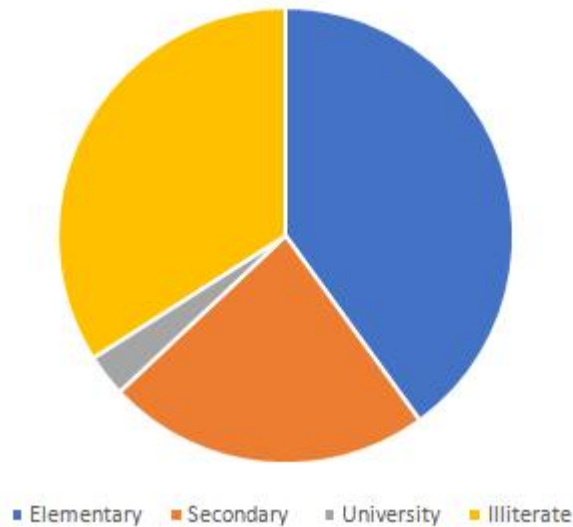
Figure 3: Distribution of the study population by level of education

Analysis of this figure shows that 40% of the study population have primary education.

Participants' knowledge of parasites

Table I shows the knowledge of the study population about intestinal parasitosis.

Table I: Study population's knowledge of intestinal parasitosis

Variables		Frequencies
Have heard of intestinal parasitosis		
Yes		77.72
No		22.28
Clinical signs of intestinal parasitosis		
Know		65.61
Don't know		34.39
Clinical signs	Diarrhoea	40.78
	Vomiting	29.12
	Abdominal pain	17.48
	Constipation	12.62
Source of contamination		
Know		77.71
Don't know		22.29
	Contaminated fruit and vegetables	13.93
Know	Dirty hands	69.67
	Contaminated drinking water	16.39

Analysis of this table shows that 157 of the 202 participants (77.72%) had heard of intestinal parasitosis. 103 (65.61%) of the 157 participants who had heard of intestinal parasitosis knew the clinical signs. 40.78% of the participants in this study who knew the clinical signs associated intestinal parasitosis with diarrhoea. Dirty hands were identified as a source of contamination by 69.67% of participants.

Parasitological examination results

Macroscopic examination

Table II shows the results of the macroscopic examination.

Table II: Stool consistency

Stool appearance	Number of participants	Percentage (%)
Diarrhoeal	07	3.47
Doughy	134	66.33
Dur	61	30.20
Total	202	100.00

66.33% of faecal samples processed here had doughy consistency.

Prevalence of parasite carriage

This is shown in the table below.

Table III: Prevalence of parasite carriage

Case	Positive	Negative	Total
Frequencies	40.10	59.90	100.00

81 of the 202 stool samples tested in this study were positive, giving an overall prevalence of 40.10%.

Parasite species detected

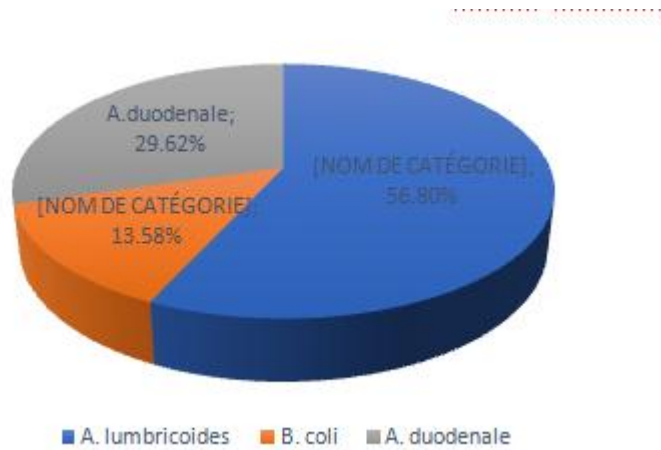Figure 4 shows the distribution of positive cases by parasite species detected.

Figure 4 : Distribution of positive cases by parasite species detected

The graph shows that *Ascaris lumbricoides* (56.80%), *Ancylostoma duodenale* (29.62%) and *Balantidium coli* (13.58%) are the three parasites species identified in the study.

Distribution of positive cases by gender

Figure 5 shows the distribution of parasitised participants by gender.
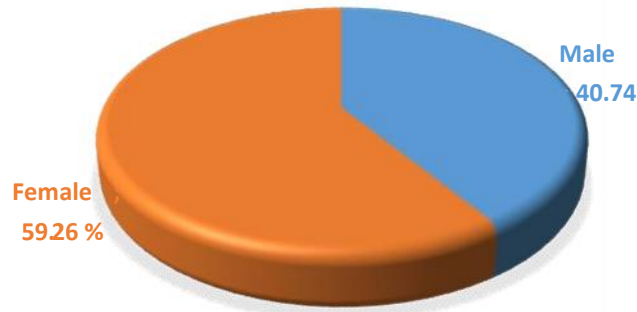

Figure 5 : Breakdown of positive cases by gender

From this figure it can be seen that more than half (59.26%) of the participants who were parasitised were female.

Age distribution of positive cases

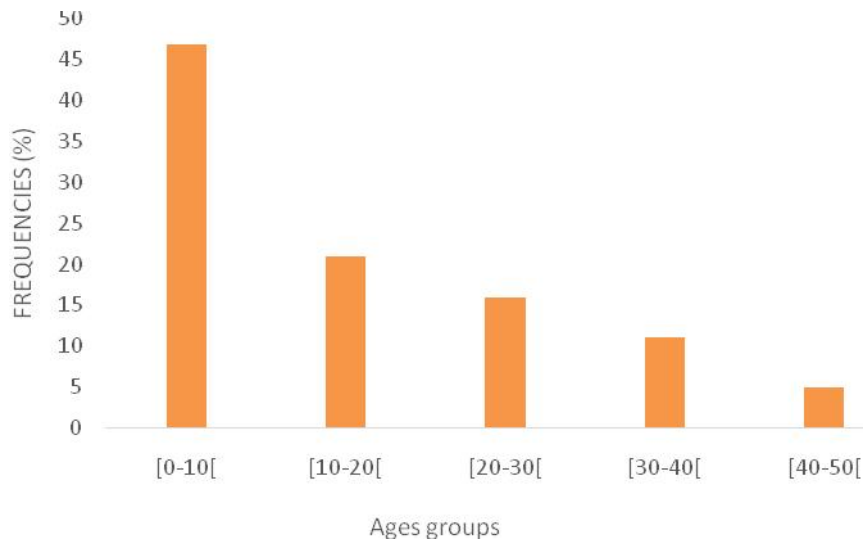The distribution of positive cases by age group is shown in Figure 6.

Figure 6: Distribution of positive cases by ages groups

This figure shows that 46.91% of parasitised participants were aged between 0 and 10 years.

The diagnostic performance of the different techniques used in this study is shown in Table IV.

Table IV: Diagnostic performance of the different techniques applied.

Techniques	Positive case	Negative case	Total
Examination with physiological water	35	167	202
Staining with Lugol's	43	159	202
Willis method	61	141	202
Ritchies method	77	125	202
Bailenger method	81	121	202

The table shows that the Willis (30.20%), Ritchie (38.12%) and Bailenger (40.10%) methods are more effective than Examination with physiological water (17.33) and Lugol's stained (21.29%).

Discussion

The aim of this study was to evaluate the diagnostic performance of the Willis, Ritchie and Bailenger methods of parasitic coprology in the Aguégués population. The study showed that out of 202 participants, more than half (59.26%) were women. This is in line with the study carried out by Frederick *et al*., in 2010, but contrary to Belhamri, who found a higher male participation in 2014. The age distribution of the study population showed that the most represented age group was 0-10 years. In this study, 66.33% of participants' stools were doughyin appearance and no adult parasites were observed on macroscopic examination. This is in line with the study conducted in Algeria by Guenidi in 2020, who reported that the most common stool appearance was pasty (75 %). At the end of this study, 81 of the 202 participants were found to be parasitised, giving an overall prevalence rate of 40.10%. This rate is still very high compared to that found in 2016 by Kansoulo in the Sèhoun district of Benin (4.20%), but lower than that of Kiki, who reported a rate of 53.12% in 2016 in the commune of Boukoumbé. This can be explained by the fact that the commune of Aguégués is a rural lakeside town with precarious environmental conditions, which are conducive to infection through the ingestion of contaminated food with dirty hands. In Kansoulo, this low rate is linked to the mass treatment organised by USAID and the deworming campaigns organised by the Sèhoun health centre, which is not the case in Boukoumbé in north-west Benin. The distribution of positive cases according to the parasitic species identified shows that *Ascaris lumbricoides* (56.80%), *Ancylostoma duodenale* (29.62%) and *Balantidium coli* (13.58%) are the three parasitic species identified in this study. Thus, 86.42% of the parasites identified by microscopic examination were helminths. This result is slightly higher than that reported by Mohamed and Mohamedi in Algeria(2017), who found a predominance of helminths at a rate of 72.3%. The predominance of helminths in the present study could be explained by the climatic conditions in the commune of Aguégués, which are favourable to the development of the helminth life cycle. On the other hand, the work carried out in 2017 by Bouragba *et al*., in Gelma, Dani and Saib in Tizi Ouzou showed the predominance of protozoa with rates of 98% and 95%, respectively. This observation is consistent with that of Sissinto-Savi de Tovè and colleagues at the Centre National Hospitalier Universitaire Hubert Koutougou Maga (CNHU-HKM) in Cotonou from 2003 to 2015, who found that the highest prevalence was observed in the protozoan class (98.1%), followed by helminths (1.9%). Microscopic examination revealed *Ascaris lumbricoides* eggs (56.80%), *Ancylostoma duodenale* eggs (29.62%) and *Balantidium coli* cysts (13.58%). The breakdown of parasitised participants by gender shows that 59.26% are female compared to 40.74% male. This result reflects the distribution of participants in this study, who are predominantly female. Compared with the literature, this rate is slightly lower than that found in Algeria in 2016 (Kasni and

Saidouni, 2016), with a rate of 60% for females compared with 40% for males. On the other hand, our results differ from those found in Constantine (Zekri and Merrouche, 2017) and Tizi Ouzou (Dani and Saib, 2017), where the proportion of parasitised subjects is higher for the male sex, with a rate of 55.70% and 63%, respectively. The distribution of positive cases by age shows that the majority of parasitised participants are children aged between 0 and 10 years, with a rate of 46.9%. This result is slightly higher than that found by Khaoula and Safa in Batna and Touggourt in 2021, where the majority of positive patients were children under 10 years of age, with a rate of 32%. This result can be explained by the more active community life of this age group and the use of river water as drinking water. We also know that children pay less attention to hygiene rules. Furthermore, our results differ from those reported in Gelma (Bouragba *et al*., 2017) and at the CHU of Oran (Benouis *et al*., 2013), where they reported higher percentages for adults with rates of 84% and 71.15%, respectively. The same is true for Zekri and Merrouche in Constantine in 2017, who found that the modal class is between 20 and 29 years of age with 35.44% of positive cases.

Regarding the techniques used, we found that the percentages of positive cases identified by Lugol's stain, Willis, Ritchie and Bailenger methods were 20.79%, 30.19%, 38.11% and 40.10% respectively, while the fresh state was used to diagnose only 17.32% of cases. Participants who were falsely negative by direct examination were corrected by concentration techniques. Concentrations techniques have increased the sensitivity of stool parasitological testing. They make it possible to concentrate in a small volume the parasitic elements initially dispersed in a large stool mass and facilitate their detection. In practice, a high-quality coprological examination combines a direct examination (fresh state/staining) and two concentrations techniques based on different principles (ANOFEL, 2016 ; Kaci *et al*., 2020). The study by Makou in 2020 showed that, from a technical point of view, concentration using the Willis and Bailenger

methods resulted in the recovery of 22 false-negative parasitological stool tests.

Conclusion

At the end of this study, it was clear that intestinal parasitosis is common in the community of Aguégués, despite deworming campaigns. Overcrowding, poor hygiene and lack of sanitation are the main factors contributing to the high incidence of intestinal parasitosis. What's more, the number of children suffering from parasitic diseases in this community is not negligible. Ascariasis, hookworm and helminthiasis are the most commonly diagnosed parasites. The Bailenger technique is proving to be more effective than the others. To combat gastrointestinal parasitosis effectively, Benin must not only systematically deworm target groups, but also organise campaigns to clean up the living environment, provide health education and sensitise medical diagnostic laboratory technicians to the use of concentration methods.

Acknowledgments

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