v. 4, n. 5, 2024

International Journal of Biological and Natural Sciences

MICROSCOPIC AND BIOMOLECULAR RESULT OF SURVEY FILARIAS DIAGNOSTIC IN THE COMMUNITY OF ANGOLA IN CHICALA PROVINCE OF BIÉ

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Abstract: Bié is referred to as an endemic location for filariasis and has never been selected for mass administration ivermectin. To understand the situation, we performed a study in the locality of Chicala, in Kuito and evaluated the co-endemicity of human filariasis, to obtain data via calibrated thick droplet microscopy and subcutaneous biopsy of samples collected in Chicala in Kuito, Bié and samples collected and preserved on Wathman filter paper for molecular diagnosis of filariae; Material and methods; The research was approved by the ethics committee of the Agostinho Neto University medical school. in deliberation number: 17/2021 and the samples collected after free and informed consent, the universe was 6015 people, data obtained from the local administration and was selected probabilistically from an approximate value of n° 400, the sample of 320 participants distributed in 12 conglomerates where we did research prospectively; The field work involved 2 microscopes, to read the slides, 2 interviewers, 1 guide and 1 driver; The *Oncocerca volvulus* worm was isolated from subcutaneous cellular tissue extracted from the iliac crest and/or calf muscles and crushed on a glass slide, hydrated in 1 drop of saline and analyzed using a binocular microscope (HC Olympus®), with a 100x objective., regarding the presence or absence of the filaria *Oncocerca volvulus* and one drop of fresh blood was collected by digital puncture and applied to the slide in a smear and on top of the slide and stained with giemsa and analyzed under the Binocular microscope (HC Olympus®) for the presence or absence of the filariae *Wuchereria bancrofty* and *Loa Loa*. All survey data were entered into Microsoft Excel 2010 and IBM SPSS version 21 statistical software was used for analysis. Results The average age of participants in the study was 39.8 years, the findings found in the calibrated thick drop were 0.3%, (+) positive for *Wuchereria*

bancrofty and subcutaneous biopsy 52.8% (+) positive for *Oncocerca volvulus*, 169/320 tests, 62.8% of participants were female, 0.3% of male participants had a positive calibrated thick drop (+) for *Wuchereria bancrofty*, no observation of the characteristic eye worm was not registered *Loa Loa* and 35% of women had a positive biopsy (+) and 17% of men had a biopsy (+) for *Oncocerca volvulus* at the age of 46-61 years, 0.3% had a positive (+) for *Wuchereria bancrofty* and, 17.5% positive (+) for *Oncocerca volvulus*, and from 14 -29 years old, 17.2%, (+) and 30-45 years old, 14.1% for *Oncocerca volvulus*. The results in the conglomerates were: Ngombakasi, 0.3% positive (+) for *Wuchereria bancrofty* and Chilomba 8.1%, Lumbachagi, 7.2%, Kalale, 6.9% and João Kapa 6.6% positive (+), for *Oncocerca volvulus*, the results of samples preserved on filter paper from the residents of Chikala are missing. Conclusions. The Chicala in the municipality of Kuito, province of Bié, is hyperendemic for *Oncocerca volvulus*, a prevalence above 50%, and hypoendemic for *Wuchereria bancrofty*, a prevalence lower than 10% with Loiasis outbreaks in the province to be determined; which allows the provincial authorities to invest in the mass administration of ivermectin because the risk of side effects is minimal or irrelevant.

INTRODUCTION

The clinical laboratory findings of co-infection *Oncocerca volvulus* and *Wuchereria bancrofty* and *Loa Loa* in Chicala, municipality of Kuito, province of Bié in a population aged between 11 and 60 years old proved the existence of co-infection, on the other hand, dried blood samples preserved on tapes filter paper, were used to confirm the microscopy results using the polymerase chain reaction as a standard test. (Van-Dúnem. P et al) Accurate diagnosis of filariasis in mobile populations can be challenging. Infected individuals may present with nonspecific symptoms or laboratory findings, and appropriate evaluation requires a strong degree of clinical suspicion as well as specialized knowledge of filarial epidemiology and pathogenesis and experience in the morphological classification of filarial parasites by microscopic examination. Further difficulties arise from the extremely limited commercial availability of diagnostic assays. of filarials that can distinguish not only between specific pathogens, but also between currently active infections and those that occurred in the past (Fink et al. 2011).

Real-time PCR assay for *O. volvulus* is significantly more sensitive than conventional microscopy for the detection of subcutaneous microfilaria. Although not is fully ready for widespread use in areas of endemicity, the successful performance of these molecular assays is an important step toward making accurate filarial diagnostic tools more accessible to clinical parasitology programs serving internationally mobile populations. (Li et al. 2011)

More sensitive tests than the search for circulating microfilariae have been increasingly used. This comes modifying Ottesen's original and relatively clear definition of the so-called "normal endemic" individuals exposed to infected mosquitoes, but with a total absence of any clinical or parasitological evidence of filarial infection, giving rise to different interpretations of diagnostic tests. In recent studies, all amicrofilaremic individuals without obvious lymphatic disease were considered normal endemic, regardless of the presence or absence of circulating antigen. Therefore, it would be necessary to follow a standard for defining the groups of individuals, whose biological samples would be tested using the different tests. With this, the results could be interpreted and compared according to the proposed objectives and the population studied. (Rocha, Áyres, and Furtado 2002)

In endemic regions where *O. volvulus*, *W. bancrofti*, *L. loa* and *M. perstans* are co-endemic, accurate and effective diagnostic tools for parasite detection are crucial to the success of any filariasis control program. In this regard, DNA-based molecular diagnosis appears to be more promising than serology, as immunological assays developed for the diagnosis or epidemiological monitoring of onchocerciasis and LF have been tested for cross-reactivity.

The ideal technique for diagnosing filarial infection in non-endemic regions is filarial real-time PCR, which has high sensitivity and specificity and is also capable of detecting a wide range of human filariae; blood samples spotted on filter paper together with saponin/Chelex 100 as a filarial DNA extraction method are a good combination for filarial epidemiological field studies in endemic countries. This method is a simple, practical and low-cost means of collecting and storing field samples and is also an economical and high-performance approach to DNA extraction. (Ta-Tang et al. 2020) Infections caused by filarial nematodes are among the most prevalent parasitic diseases worldwide. Although transmission of these organisms is geographically restricted to areas of developing countries where the disease is endemic, modern human travel patterns have resulted in the migration of infected individuals to regions where filarial infections have been eradicated or were never present, including resource-rich regions. just like the United States. Although they are relatively infrequent, filarial infections are sporadically diagnosed in refugees and other immigrants from endemic areas, in long-term residents of regions where filarial regions are endemic (members of the armed forces, students, missionaries, aid workers and volunteers), and, rarely, among short-term travelers (Li et al. 2011)

The general objective of the present study is to compare the findings of molecular diagnosis and microscopic diagnosis of filariae by calibrated thick drop and biopsy of a research carried out in Chicala, municipality of Kuito, province of Bié.

MATERIAL AND METHODS

Research approved by the faculty ethics committee of medicine from Agostinho Neto University. in deliberation number: 17/2021 and the samples collected after free and informed consent and population data received from the local administration. The study involved 2 microscopists, to read the slides, of 320 residents from a universe of 6015 people from 12 conglomerates selected probabilistically from an approximate value of (n°) 400, which resulted in a sample of 320 people who participated in the research prospectively; The *Oncocerca volvulus* worm was isolated from subcutaneous cellular tissue extracted from the iliac crest and/or calf muscles and crushed on a glass slide and hydrated in 1 drop of saline solution and analyzed with a binocular microscope (HC Olympus®), with a 10x objective., regarding the presence or absence of the filaria *Oncocerca volvulus* and 1 drop of fresh blood was collected by digital puncture and applied to the smear and on top of the coverslip and stained with giemsa and analyzed under a Binocular microscope (HC Olympus®) for the presence or absence of the filaria *Wuchereria bancrofty* and *Loa loa*. and 320 dried blood samples on Whatman filter paper were collected It is preserved for processing of microfilariae concentration; for DNA extraction from the dried blood stain and used positive lamina for *L. loa*, *W. bancrofty* and *Oncocerca volvulus* from patients from endemic communities in Angola used as a positive control to compare with the result of the positive filter paper. DNA extraction from the dried blood spot

preserved on Whatman filter paper was obtained with the QIAamp ® Mini DNA Kit (QIAGen), according to the manufacturer's instructions. (Rubio et al. 2002). All survey data were entered into Microsoft Excel 2010 and IBM SPSS version 21 statistical software was used for analysis.

DNA EXTRACTION

The extraction of DNA from the dried blood sample on Whateman filter paper of *Loa Loa*, *W. bancrofty* and *Oncocerca volvulus* and compared with positive samples from patients from endemic communities in Angola and used as positive controls and negative samples from *W. bancrofty, Loa loa* and *Oncocerca volvulus*, used as a negative control, according to (Brito et al. 2020).

Material

Microfilariae samples

Lysis buffer solution (e.g. lysis buffer containing SDS and proteinase K Phenol-chloroform

Cold ethanol

TE Buffer (Tris-EDTA) Centrifuge

Microcentrifuge tubes Sterile pipettes and tips Incubator at 56ºC

latex gloves

Sample Preparation

Collection of filaria and transfer to the Cell Lysis microcentrifuge tube:

Added 500µl of lysis buffer solution to the sample Added 20µl of proteinase K (20mg/ml)

Incubated at 56ºC for 1-3 hours until complete sample lysis

Extraction

Added 500µl of Phenol Chloroform

Homogenize the mixture and centrifuge at 10,000g /10 min Transfer the aqueous phase to the new microcentrifuge tube

DNA precipitation

Added 2 volumes of ice-cold ethanol to the aqueous phase Mixed gently and incubated at 20ºC for 1 hour Centrifuged at 10000g / 10 min to pellet the DNA

DNA Pellet Washing; The supernatant was carefully discarded

Wash the DNA pellet with 50µl of ice-cold 70% ethanol Centrifuge again at 10000g / 5 minutes. Discard the supernatant and allow the pellet to dry freely DNA resolubilization.

Suspend the DNA pellet in 50-100µl of buffer incubated at 37ºC for 1 hour to ensure complete dissolution of the DNA.

The extracted DNA was stored at -20ºC, the purity of the DNA using spectrophotometry was in the A260/A280 ratio and was 1.8 and 2.0, the extraction was carried out under sterile conditions and pipettes and filter tips were used to manipulate the reagents of DNA extraction from filariae using QIAamp®DNA Mini Kit and Chelex Method.

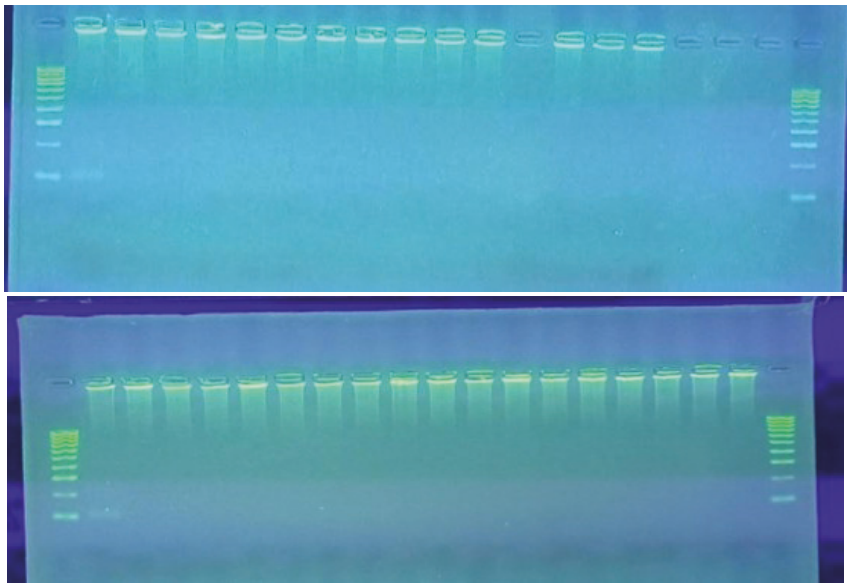
INTRODUCTION

Representation of the DNA extraction process from *Wuchereria bancrofty*, *Loa Loa* and *Oncocerca volvulus* and results

DNA EXTRACTION (PROCEDURE)

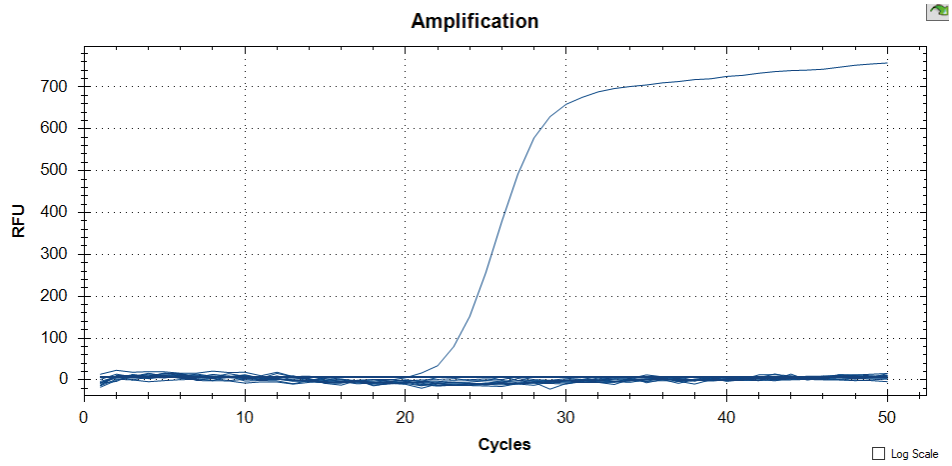
Collection of dried blood samples on Watheman filter paper

Cell lysis with the QIAamp ® DNA Mini kit following the manufacturer's instructions DNA extraction and purification of 2% agarose gel for *Loa Loa* research, where the positive control band can be seen.



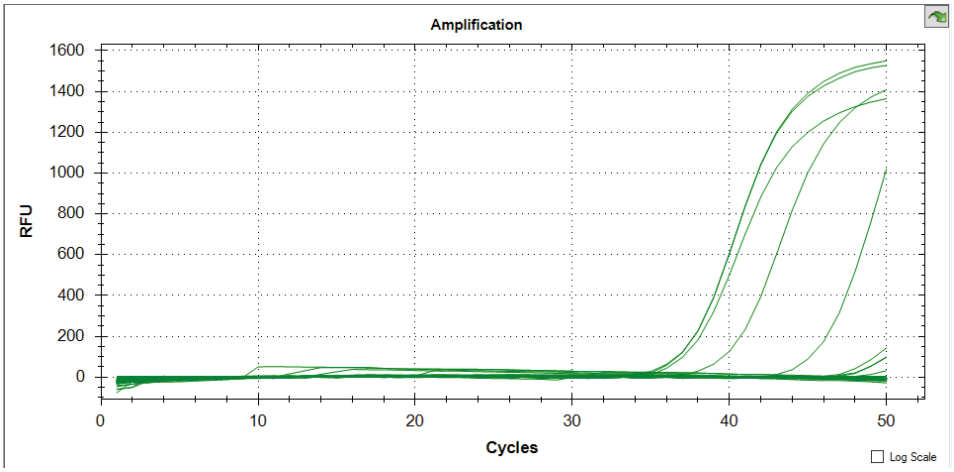
Real-time PCR amplification curve for *Wuchereria bancrofty*

The only sample that amplifies is the positive control

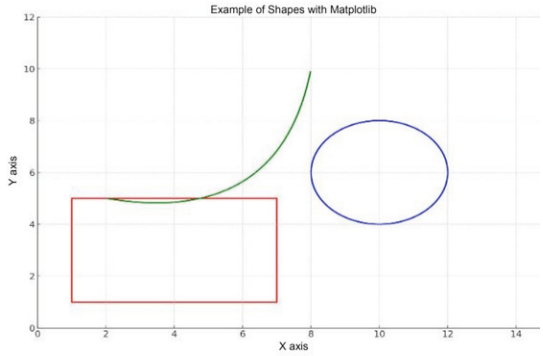


Real-time PCR amplification curve for *Oncocerca volvulus*

Several positive samples and the positive control are marked



Representation in shapes geometric as It is View per Complex data in a simplified and visually understandable way the results *Wuchereria bancrofty* (320/320) (-) *Loa Loa* (320/320) (-) and *Oncocerca volvulus* (15/320) (+)

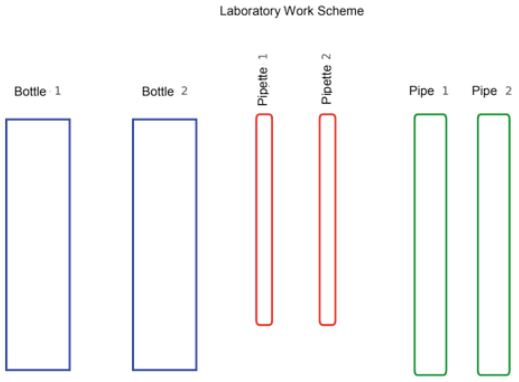


DNA EXTRACTION PROCESS FROM *WUCHERERIA BANCROFTY, LOA LOA* AND *ONCOCERCA VOLVULUS*

DESCRIPTION OF LABORATORY WORK

BOTTLES

Bottle



Represented in a blue rectangle located in position (1,1) with a figure of 2 units and a height of 5 units

Bottle 2: Represented in a blue rectangle located in position (5,1) with the same dimensions as bottle 1

PIPETTES

Pipette 1: Represented by a red rounded box located at position (9,2) with a width of 0.3 units and a height of 4 units, rotated vertically

Pipette 2: Similar to Pipette 1 but located in position (11,2)

RESULTS

(% calculated taking into consideration, the sample n=320)

The average age of participants in the study was 39.8 years, 95% confidence interval between 38.0 and 41.6 years, and the standard deviation was 16.5 years, the minimum age was 14 years and the maximum age was 90 years, we can say that there is no great dispersion in ages. The median showed that 50% of participants were over 38 years old

Statistical		Value
Average		39.8
Confidence interval	inferior limit	38.0
	upper limit	41.6
Median		38.0
Standard deviation		16.5
Minimum		14
Maximum		90

Table 1. Statistics on the age distribution of Chicala residents

Source: author details

The results of the tests carried out were 0.3% positive (+) in the calibrated thick drop and 52.8%. Positive (+), corresponding to 169/320 subcutaneous biopsy tests performed.

Tests	Result			
	Negative		Positive	
	Number	%	Number	%
Calibrated E. Drop	319	99.7	1	0.3
Biopsy	151	47.2	169	52.8

Table 2: Distribution of test results carried out among Chicala residents

Source: author details

Regarding test results by gender, 62.8% of participants were female, 0.3% of male participants had thick gout calibrated positive (+) for *Wuchereria bancrofty* with a significance of 0.3, there was no observation of the ocular worm characteristic of *Loa Loa* in relation to the results of the subcutaneous biopsies performed 35% of women had a positive biopsy (+) and 17% of men had a biopsy (+) with a significance of 1.

Regarding test results by age group, 46-61 years old, 0.3% had a positive calibrated thick drop (+) and significance of 0.5 and for biopsy the 46-61 age group, 17.5% had a positive test (+), in the 14-29-year-old range, 17.2%, tested (+) and in the 30-45-year-old range, 14.1% and significance was 0.

In relation to the results of the tests by localities, Ngombakasi had 0.3% of thick calibrated droplet positive (+) for *Wuchereria bancrofty*, the significance was 0.4, in relation to the subcutaneous biopsy Chilomba had 8.1%, Lumbachagi, 7.2%, Kalale, 6.9% and João Kapa with 6.6% of positive subcutaneous biopsies performed (+)

Parasite (Filaria)	PCR Result			
	Negative		Positive	
	At the	%*	At the	%*
W. bancrofty	320	100.0	0	0.0
O. volvulus	302	94.4	18	5.6
L. loa	320	100.0	0	0.0

Table 6: Distribution of PCR results by disease in Chicala Kuito Province of Bié

*The percentage was calculated taking into consideration, the total number of respondents n=320

Source: author details

DISCUSSION

This was not the first research on filariae in the province of Bié to determine the co-endemicity of filariae. This research used microscopic diagnosis as the main tool, although physical examinations were carried out in the field, including examination of the fundus of the eye with flashlight, To obtain an important amount of information, important in determining the co-endemicity, in relation to the age group of the participants, it was important to relate the incubation period of filariasis, which varies from 5 years for *Oncocerca volvulus* and 15 years for *Wuchereria bancrofty*(Coura 2015; Falcão 2002; Rey 2019) with the average age of research participants in Chicala being 39.8 years old with a 95% confidence interval between the ages of 38 and 41.6, on the other hand our confidence interval is 0.3% in the thick drop for the age group rejects the null hypothesis, i.e. shows no evidence for the null hypothesis. the results showed that the chance of finding positive results either in the subcutaneous biopsy or in the calibrated thick drop is 95%. On the other hand, the results presented were determined by microscopy and for *Wuchereria bancrofty* the clinical findings were not confirmed laboratory-based. During fieldwork we found participants from Chicala with elephantiasis, lymphedema and hydrocele, but the microscopy results (GEC) were negative and the prevalence of *Wuchereria bancrofty* was 0.3% lower than those recorded in the results of the strategic plan for Tropical Diseases neglected, which ranged from 0.5% to 8% (DNSP and MINSA 2022). These results can be justified by the use of the Alere® immunological test (FTS), while we only used the calibrated thick drop, and also the results of our study may be influenced by the periodicity (Fontes et al. 2008; Ministry of Health 2010; Thompson et al. 1996) characteristic of *Wuchereria bancrofty*, as the

Gender	Total		Calibrated Thick Drop				Statistical significance (Chi Square Test)	Subcutaneous Biopsy				Statistical significance (Chi Square Test)
			(-)		(+)			(-)		(+)		
	Number	%	Number	%	Number	%		Number	%	Number	%	
Masculine	119	37.2	118	36.9	1	0.3	0.37	62	19.4	57	17.8	0.18
Feminine	201	62.8	201	62.8	0	0.0		89	27.8	112	35.0	

Table 3. Distribution of test results carried out by sex in the town of Chicala

Source: author details

Age range	Total		Calibrated Thick Drop				Statistical significance (Chi Square Test)	Subcutaneous Biopsy				Statistical significance (Chi Square Test)
			(-)		(+)			(-)		(+)		
	Number	%	Number	%	Number	%		Number	%	Number	%	
14-29	95	29.7	95	29.7	0	0.0	0.59	40	12.5	55	17.2	0.00
30-45	110	34.4	110	34.4	0	0.0		65	20.3	45	14.1	
46-61	84	26.3	83	25.9	1	0.3		28	8.8	56	17.5	
62-77	26	8.1	26	8.1	0	0.0		18	5.6	8	2.5	
78 and over	5	1.6	5	1.6	0	0.0		0	0.0	5	1.6	

Table 4: Distribution of test results carried out by age group in the town of Chicala

Source: author details

Household	Total		Calibrated Thick Drop				Statistical significance (Chi Square Test)	Subcutaneous Biopsy				Statistical significance (Chi Square Test)
			(-)		(+)			(-)		(+)		
	Number	%	Number	%	Number	%		Number	%	Number	%	
In	23	7.2	23	7.2	0	0.0	0.41	12	3.8	11	3.4	0.00
Chilomba	29	9.1	29	9.1	0	0.0		3	0.9	26	8.1	
Kapamba	27	8.4	27	8.4	0	0.0		23	7.2	4	1.3	
João Kapa	24	7.5	24	7.5	0	0.0		3	0.9	21	6.6	
Ngombakasi	26	8.1	25	7.8	1	0.3		17	5.3	9	2.8	
Kalale	28	8.8	28	8.8	0	0.0		6	1.9	22	6.9	
Sagombo	26	8.1	26	8.1	0	0.0		16	5.0	10	3.1	
Lumbachagi	27	8.4	27	8.4	0	0.0		4	1.3	23	7.2	
Yeyele	26	8.1	26	8.1	0	0.0		12	3.8	14	4.4	
Epomba	26	8.1	26	8.1	0	0.0		16	5.0	10	3.1	
Kaninguir	26	8.1	26	8.1	0	0.0		17	5.3	9	2.8	
Benbua	32	10.0	32	10.0	0	0.0		22	6.9	10	3.1	

Table 5: Distribution of test results carried out by neighborhoods or Kimbos in the Chicala locality

Source: author details

	Sex	PCR Result				Total[Neg (-)]	
		Post (+)					
		At the	%*	At the	%*	At the	%*
Sex	male	112	35.0	7	2.2	119	37.2
	female	190	59.4	11	3.4	201	62.8
Age	14-29	88	27.5	7	2.2	95	29.7
	30-45	104	32.5	6	1.9	110	34.4
	46-61	80	25.0	4	1.3	84	26.3
	62-77	26	8.1	0	0.0	26	8.1
	78 and more	4	1.3	1	0.3	5	1.6

Table 7: Distribution of PCR results according to sex and age group in Chicala Kuito Province of Bié

*The percentage was calculated taking into consideration, the total number of respondents n=320

Source: author details

samples studied were collected from 8 am to 3 pm, a period in which the chance of finding positive results is lower, which is why our calibrated thick drop measurement was 0.3%, for a confidence interval of 0.4%, allowed us to state that the results obtained in the calibrated thick taste reject the null hypothesis; As for the biopsy, which we measured at 52.8%, for a confidence interval of 0.0 the results show evidence for the null hypothesis. The result of Chicala leads us to reflect on the coeicity of filariae, in addition. Mapping the community prevalence of filarial antigen detected with the Immunochromatographic Rapid Diagnostic Test (ICT) Card (Binax NOW Filariosis), and now, more recently, with the Filariasis Test Strip (FTS)(Weil et al., 2013,Weil and Ramzy, 2007). (B. Brito et al. 2017) indicate a problem of cross-reactivity with the ICT card in *L. loa* areas resulting in false positives and potentially an overestimation of FL prevalence (Bakajika et al., 2014,Pion et al., 2016b,Wanji et al., 2015,Wanji et al. 2016(Bockarie et al. 2015; Gounoue-Kamkumo et al. 2015; M. Brito et al. 2017) calibrated

In relation to *Oncocerca volvulus*, the first mapping carried out in Angola took place in 2002 using the REMO technique, carried out with support from the African Program to Fight Onchocerciasis (APOC). And 114 villages were evaluated that year and 421 communities were subsequently evaluated by subcutaneous biopsy in 2011. The result of the data analysis identified 367 villages; (DNSP and MINSA 2022) in 2015, 76 villages were mapped, using subcutaneous biopsy to diagnose cases. The integration of the results of the two mapping exercises (REMO and subcutaneous biopsy) indicated that onchocerciasis was endemic in 48 municipalities of the 12 provinces where the tests were carried out, because of the results and rumors, and even the record of the presence of heartworm eye (*Loa Loa*) in the province of Bié, the ADM was postponed.

The study revealed a prevalence of 52.8% by subcutaneous biopsy, in the town of Chikala and the presence of young blind people, and depigmentation on the lower limbs in several people, which is a sign resulting from subcutaneous lesion, caused by itching. Among the positive results we even found in traditional authorities, so we can classify the epidemiological situation of onchocerciasis in that locality as hyper endemic according to the stratification of the endemicity of onchocerciasis (Silva 2015). While in *Loa Loa*, no positive finding was found in the calibrated thick drop.

The generated image can be understood in a visual analogy of the representation of data related to filaria in two main aspects the image in geometric shapes is the way it is seen and represented by complex data in a simplified and visually understandable way. In the context of filariae, which are parasites that cause diseases such as filariasis, data visualization is crucial to understand geographic distribution, life cycles, infections and control interventions. Because the rectangle can represent affected geographic areas, circles can indicate infected populations and curved arrows can illustrate transmission between hosts or the life cycles of parasites. Filariae such as those that cause lymphatic filariasis such as *Wuchereria bancrofty* or onchocerciasis such as (*Oncocerca volvulus*) have cycles in complex life that can be illustrated using diagrams similar to the one generated. Rectangles can represent different stages of the life cycle (e.g. larvae in mosquito, adult stage in humans), circles can denote populations of vectors or infected humans, and arrows can illustrate movement of parasites between different stages or hosts.

CONCLUSIONS

The result of microscopic diagnosis by calibrated thick drop was (0.3%) positive (+) and (52.8%). positive (+) for subcutaneous biopsy and molecular diagnosis of filaria in samples collected in the commune of Chicala, province of Bié.

The evidence from our results shows that the locality of Chicala is hyperendemic for *Oncocerca volvulus* and hypoendemic for *Wuchereria bancrofty* and without loiasis;

With these results, we encourage the authorities in the town of Chicala to invest in the strategy of mass administration of ivermectin without the risk of side effects arising from its use.

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