Review Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 26/03/2024;
Date Revised: 26/11/2025;
Available Online: 28/12/2025;

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How to Cite:
Said NM, Nisha M (2025). A review on anthelmintic resistant markers in *Ascaris lumbricoides*. Adv. Life Sci. 12(4): 642-650.

Keywords:
Anthelmintic resistance; *Ascaris lumbricoides*; β-tubulin gene

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A review on anthelmintic resistant markers in *Ascaris lumbricoides*

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Abstract

Ascaris llumbricoides is a soil transmitted helminths (STH) found in many developing countries affecting about 820 million people worldwide. Anthelmintic drugs recommended by the World Health Organization (WHO) are used to treat the STH infections due to their low cost and high efficacy against these parasites. Despite the treatment efforts, *Ascaris lumbricoides* remains an endemic issue sparking concerns about the parasite's resistance to the anthelmintic drugs. This study aims to review the reports on the presence of single nucleotide polymorphism (SNP) of β-tubulin gene of codon 167, codon 198 and codon 200 that is associated with anthelmintic resistance in *Ascaris lumbricoides*. Only studies published between 2009 to 2020 were included to maintain the relevance of the findings from these publications to fit the criteria set. The reported prevalence of *Ascaris lumbricoides* pre-treatment and post-treatment was collected and compared. The method or protocol done for DNA extraction and gene amplification was considered to the possible difference in results. The presence of SNPs detected in codon 167, codon 198 and codon 200 of β-tubulin gene of *Ascaris lumbricoides* is recorded in any reported polymorphism or heterozygous resistant gene. Molecular method that was used in studies that showed positive results were also recorded to compare the most suitable method in detecting SNPs of codons that are related to resistance in *Ascaris lumbricoides.* Seven publications from previous years ranging from 2009 to 2020 were reviewed, discussing the anthelmintic resistance markers found in *Ascaris lumbricoides* show evidence of the presence of SNPs in codon 167 and codon 200 of the β-tubulin gene. It can be concluded that the presence of these polymorphisms linked to anthelmintic resistance influences the efficacy of anthelmintics towards *Ascaris lumbricoides.*

Introduction

Ascaris lumbricoides is a type of parasitic worm classified as soil-transmitted helminths (STH) and is often found in many developing countries. About 820 million people worldwide are infected with *Ascaris lumbricoides* which proves its high prevalence [1]. *Ascaris lumbricoides* enters the body either when a person ingests food or water that are contaminated with the eggs of *Ascaris lumbricoides* or contact with contaminated soil [2].

Anthelmintic drugs like benzimidazoles (BZ), piperazine and pyrantel are recommended by the World Health Organization (WHO) in treating STH infections as it is low-cost and has a quite high efficacy against them. Through mass drug administration (MDA), inexpensive medicines with the principles of preventative chemotherapy are distributed to targeted populations where treatments are offered without any initial diagnosis. MDA helps in reducing the symptoms and morbidity, as well as reducing the transmission of neglected tropical diseases (NTDs) in endemic areas [3]. Despite this, *Ascaris lumbricoides* remains an endemic issue, raising concerns about the development of resistance towards BZ [2,4]. The resistance developed on STH are often caused by single nucleotide polymorphism (SNP) of the β-tubulin gene on codon 167, codon 198 and codon 200 [5]. According to previous studies, the existence of SNPs of codon 167 and codon 198 were discovered in *Ascaris lumbricoides*. This suggests the possible development of resistance of *Ascaris lumbricoides* towards anthelmintics [2,6].

Methods

Search strategy

Search engines like Google Scholar, PubMed, Elsevier, Scopus, NCBI and Science Direct were searched for keywords relating to the title. Keywords that were used to search the appropriate papers included Soil-transmitted helminth, *Ascaris*, *Ascaris lumbricoides*, ascariasis, anthelmintic, benzimidazoles, benzimidazole resistance, albendazole, mebendazole, ivermectin, anthelmintic resistance and Single-nucleotide polymorphism. Only studies ranging from the year 2009 to 2020 were considered to maintain the relevance of discovery from these publications. Collected articles were screened for the inclusion of data based on 3 criteria; i) Researches with primary data on presence of β-tubulin gene resistant in *Ascaris lumbricoides*. ii) Studies that compare the prevalence of *Ascaris lumbricoides* pre- and post-treatment of anthelmintic. iii) Study population that has been included in their government's local MDA programmes or received anthelmintic treatment sometime during the study.

Data extraction

Data were extracted from each article regarding the country, study year, location, population, age group, number of samples collected and types of anthelmintic used in treatment. The reported prevalence of *Ascaris lumbricoides* pre-treatment and post-treatment was collected and compared. The methods or protocols done for DNA extraction and gene amplification were considered as possible factors relating to differences in results. The data regarding the absence or presence of polymorphism in codon 167, codon 198 and codon 200 was reviewed along with the primer sequence used.

Data analysis

The presence of SNPs detected in codon 167, codon 198 and codon 200 of β-tubulin gene of *Ascaris lumbricoides* is recorded in any reported polymorphism or heterozygous resistant gene. Molecular method that was used in studies that showed positive results were also recorded to compare the most suitable method in detecting SNPs of codons that are related to resistance in *Ascaris lumbricoides*.

The extracted data were qualitatively analysed and compared across studies. SNP occurrence, study location, sample characteristics, and treatment history were reviewed to determine potential relationship between molecular markers and anthelmintic resistance. The molecular detection techniques were evaluated in terms of their effectiveness and frequency of use to identify methodological trends among the reviewed publications.

This review compared study findings, molecular methods, and study conditions such as anthelmintic type and population to summarize available evidence on β-tubulin gene polymorphisms. The analysis aims to identify consistent results, methodological differences, and remaining gaps in understanding anthelmintic resistance in *Ascaris lumbricoides.*

Discussion

After inserting keywords into the search engines, only thirteen studies that fit the criteria to the topic were shortlisted and reviewed. However, after further review, 4 of these studies did not include the polymorphism at codon 167, codon 198 and codon 200 of the β-tubulin gene in *Ascaris lumbricoides*. Instead, they focused more on the cure rate (CR) and egg reduction rate (ERR) for the prevalence of *Ascaris lumbricoides*. Two other studies researched more on the comparison of different techniques used to amplify the β-tubulin gene rather than researching a population that could show the presence of polymorphism. Hence with the exclusion of these studies, the total full-text articles that are included in this review amounted to seven articles.

These seven articles were confirmed for its legitimacy and publication.

An article that is included in this review studied the polymorphism in the β-tubulin gene of *Ascaris suum* isolated from livestock, a species that has been deemed the same species as *Ascaris lumbricoides* due to the similarity in their genetic makeup and distinguished only by their zoonotic nature. In fact, in that study, a crossbreed between the pig and human-derived *Ascaris* species was discovered. With other publications confirming the evidence of the two species as the same [7], this article was included in the study. A list of all the articles reviewed is as shown in Table 1. The countries included in this review are Honduras (2 publications), Haiti, Kenya, Panama, Rwanda, Brazil (2 publications), Zanzibar and Uganda. Another 2 publications studied and compared the results between multiple countries.

Study age group and sample size

The sample size and number of genotyped samples in these publications vary and do not show a clear range that particularly shows the best outcome. The differences between the sample size and specimens genotyped ranges significantly. School children were the more common participants in the studies, with a total of 6 from 7 studies (85.7%). Specimens that are collected from the participants can be either adult worms or eggs collected from fecal sample.

Anthelmintic history of participants

In most of the studies, anthelmintic was distributed to the participants at some point of the study. A single dose of albendazole (400mg) is the most common anthelmintic used in this study. Pyrantel and piperazine was also seen distributed among the participants, which leaves mebendazole and ivermectin excluded from these studies (Figure 1).

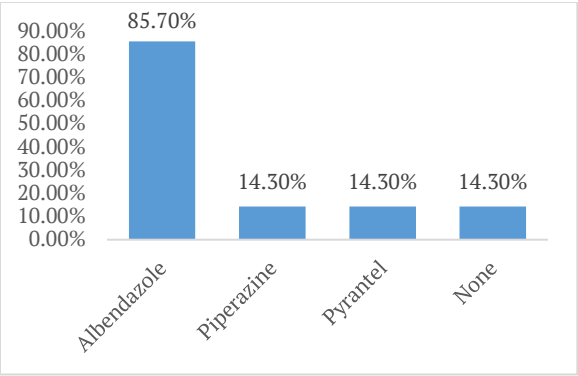

Figure 1: Type of anthelmintic used in the studies showing that albendazole is more commonly distributed to the study participants.

Meanwhile, the majority of participants in these studies had been involved in their local MDA programmes. These programmes are frequently held to control STH infections in area with high prevalence, particularly in rural regions. From the 11 study cohorts examined in these 7 publications, 8 had undergone MDA, 2 were naïve of albendazole and 1 had no documented record of involvement in an MDA programme. Following WHO guidelines, MDA is done with benzimidazole, either albendazole or mebendazole. However, all MDA programmes found in these reviewed studies uses albendazole.

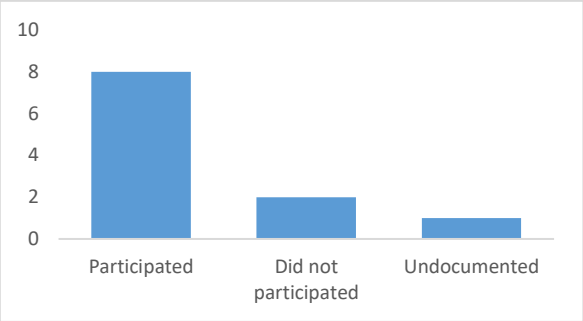

Figure 2: Study participant's prior involvement in MDA programmes which are held to control STH infections.

For the research that was done on participants that did not undergo MDA, they received anthelmintic during the duration of the study. The 2009 study by Aissatou Diawara and her colleagues had the participants in Kenya, naïve of anthelmintic history. They received one dose of combantrin (pyrantel) before sample collection. Meanwhile in Panama, it was not documented whether the participants have received any anthelmintic prior to the study. It is surmised that they may have had received anthelmintic at some point before the study however it is only speculation of the author.

A 2013 study done by the same author, Diawara and her colleagues saw that the participants of the study in Haiti has never received anthelmintic through MDA programmes. Samples were taken before albendazole distribution and 2 weeks after administration.

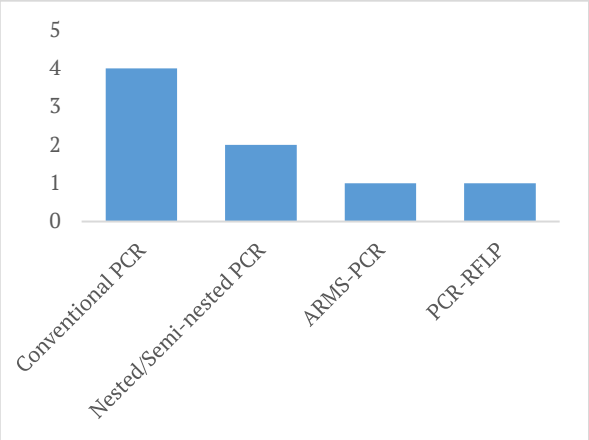

Figure 3: Frequency of molecular technique used to analyse presence of polymorphisms in codons linked to anthelmintic resistance.

Molecular technique used for resistance analysis

For DNA amplification, different types of PCR were used for the analysis. The different PCR method used could play a major factor in the presenting of the results in these studies. The molecular techniques seen in these studies includes conventional PCR, Semi-nested/nested PCR, ARMS-PCR and PCR-RFLP. The molecular technique that showed positive results for the presence of polymorphism in codons associated with anthelmintic resistance in *Ascaris lumbricoides* are conventional PCR and ARMS-PCR.

Presence of SNPs on Codon 167, 198 and 200 of β-tubulin gene

All of the publications reviewed analysed codon 167, codon 198 and codon 200 with the exception of a study by Zuccherato and his colleagues in 2018 that analysed codon 167 and codon 198, while a subsequent study done by Furtado and his colleagues in 2019 analysed codon 200. These two studies had the same study demographic and shared the collected specimen, differed only by its analysis of codons related to anthelmintic resistance. The presence of anthelmintic resistance is determined by the detection of polymorphism at codon 167 and 200 that encodes for phenylalanine (TTC/TTT), and codon 198 that encodes for glutamic acid (GAG/GAA). The results of studies on the presence of polymorphism in these codons are shown in Table 3.

Result Analysis

In the present study, the presence of anthelmintic resistant markers in *Ascaris lumbricoides* was reviewed. Studies regarding polymorphism related with anthelmintic resistance specifically in *Ascaris lumbricoides* are very limited with only 7 publications that matched the criteria of this review.

From the review, the 8 regions that were included in these publications consists of Haiti, Kenya, Panama, Honduras, Zanzibar, Rwanda, Uganda and Brazil. All regions that participated in these studies were reported as rural and poor sanitation areas. According to the United Nations (UN) in 2021, Haiti, Uganda, Zanzibar and Rwanda are categorized as a part of low development countries (LDC) based on their human development index (HDI) where a HDI index below 0.550 is deemed as a least developed country. Honduras and Kenya are both very close to the baseline of the index, where the HDI is 0.617 and 0.601 respectively. STHs thrive in areas with poor sanitation and water supply [8]. This is the case for the LDC because due to poor hygiene and water contamination, their populations are constantly exposed to the risk of various infections, including STHs related infections. Meanwhile, Brazil (0.759) is considered as a country with high human development and Panama (0.815) considered as a country with very high human development. Despite having high human development, STHs incidence are still high in these countries [9]. Since rural areas of these countries are less developed than their urban counterparts, lower water quality and hygiene is the highest contributing factors to STHs infections.

The age group of these studies as shown in Table 2 shows that all the publications included children, apart from the 2020 study by Palma and his colleagues where the samples were taken from domestic pigs. Children of the ages 3 to 8 years old are especially susceptible to being infected with *Ascaris lumbricoides* [10] which justifies these publications selection of children as their participants. Adults are also seen in 3 out of 7 studies where they are included together with the children as participants. As *Ascaris lumbricoides* can infect people of various age, it is reasonable for the studies to include adults as participants of their studies. As for the sample size, it varies between each of the studies and there was no mean/average size that would imply the perfect sample size that should be done for this study. The sample size ranges from 8 children to 353 children, with Kenya having an unspecified sample size in the study done by Diawara and her colleagues in 2009. The amount of specimen that were genotyped from the sample size also differs from each study. Each participant from these studies may provide more than one adult *Ascaris lumbricoides* worm or egg. The most severe cases are the study done in Honduras by Matamoros in 2019, where 8 children that are severely infected with STHs provided 452 samples that were genotyped to be investigated for its polymorphism.

Most of the studies opted to distribute albendazole as a part of their research at 85.7% of the studies using it. Albendazole is very widely used, including in preventative chemotherapy and MDA. WHO guidelines states that preventative chemotherapy or deworming, are to be done with benzimidazole, either albendazole (400mg) or mebendazole (500mg) or pyrantel (10mg/kg) and should be done yearly or twice a year. While albendazole is prominently used in these publications, none of these studies has used mebendazole. It can be observed that the efficacy of albendazole in treating ascariasis is greater than mebendazole [11]. Meanwhile, pyrantel and piperazine are known anthelmintics to treat against STH but are less commonly used in this period of time. Pyrantel are still being used in MDAs but benzimidazole drugs are being made available to WHO by the donations of pharmaceutical companies. These donations lead to the more prominent use of benzimidazole in treating ascariasis, as opposed to other

anthelmintic that is proven to be efficacious against *Ascaris lumbricoides* [12]. The study that uses piperazine has incorporated it with the administration of albendazole to amplify the effects of the drugs as the participants were heavily infected with STHs. Piperazine alone is rarely seen used for treatment of ascariasis, in fact, now it is a more common drug in veterinary medicine [13]. Piperazine citrate was more frequently used in the early 20th century, before benzimidazole was created. Since then, piperazine was discovered to be less efficient in combating *Ascaris lumbricoides* [14], hence why benzimidazoles and other anthelmintic is more preferred.

Figure 2 shows that the majority of the studies' participants (72.7%) have participated in MDA programmes of their respective regions. The drugs administered for the MDA programmes were not documented but was confirmed with the government of each country. Generally, drugs that are distributed in the MDA programmes are usually albendazole [15]. 18.2% of the participants were naïve of anthelmintic and has never participated in any MDA programmes while 9.1% of the participants' MDA history was undocumented. However, the author speculates that at some point prior to the study, the participants have received anthelmintic. Participants that were naïve of any consumption of anthelmintic was distributed a type of anthelmintic during the period of the study in an attempt to observe the development of resistance in *Ascaris lumbricoides*. Having a history of anthelmintic consumption is very significant in this study, as it gives the helminths opportunities to develop resistance, prior to the studies conducted.

From the reviewed studies, there was no set of standards for the DNA amplification procedures. Around 57.1% of the studies implemented conventional PCR as their DNA amplification method. Meanwhile, 28.6% used a nested or semi-nested PCR, 14.3% used a modified PCR-RFLP technique, and 14.3% used ARMS-PCR with some studies employing more than one technique, as a result, the percentages reflect the overall frequency of each method's use rather than mutually exclusive study counts. Restriction fragment length polymorphism (RFLP) has a high specificity and is used to further localize the SNPs relating to anthelmintic resistance in *Ascaris lumbricoides*. It works by cleaving the restriction enzyme sites that are present in the gene. ARMS-PCR detects mutations at single base pairs and is highly sequence-specific [16]. All of the techniques are used with primers that were specific to the codon position of interest. The two techniques that identified the presence of anthelmintic resistant related marker are the conventional PCR and ARMS-PCR. Conventional PCR detected polymorphism on only codon 167 and ARMS-PCR detected the presence of polymorphism on codon 200 of the β-tubulin gene of *Ascaris lumbricoides*. This suggests that ARMS-PCR and conventional PCR are excellent techniques in the approach of detecting the SNPs on codon 167 and 200. Based on these publications, the only method to detect the anthelmintic resistance in *Ascaris lumbricoides* in the molecular level is by detecting the variation or polymorphism on codons that are linked to anthelmintic resistance. Other molecular traits that can be linked with anthelmintic resistance, such as the presence of drug receptors that causes restriction of drug efficacy and the helminths metabolic pathways that could possibly regulate the amount of drug that is passed by the helminths [17] are not explored in publications relating to *Ascaris lumbricoides* anthelmintic resistance. The study of anthelmintic resistance in *Ascaris lumbricoides* on the molecular level is relatively new with the first study reported by Diawara and colleagues in 2009. This study was done following the discovery of polymorphism on codons of the β-tubulin gene relating to anthelmintic resistance in *Haemonchus contortus* which is why the possibility of other molecular diagnosis on this matter Is possible to be applied to the study in ascaris lumbricoides.

Overall, from the findings of the reviewed articles, only 28.57% have produced a result that identified the presence of anthelmintic resistance relates marker in *Ascaris lumbricoides* (Table 3). This represents a relatively low percentage considering the number of samples collected in each study is very high. In 2013, Diawara and her colleague discovered the presence of polymorphism in codon 167 using conventional PCR as the DNA amplification technique. The sample that showed this result came from Haiti where the sample size is significantly larger than the other region studies by Diawara which could factor into this discovery. However, data collected regarding the egg reduction rate (ERR) for this region strongly suggests that the treatment was successful parallel to WHO standards. This ERR data implies that the presence of SNPs in B-tubulin codon 167 in *Ascaris lumbricoides* had no effect in the efficacy of ABZ. The presence of SNPs on codon 200 of β-tubulin gene of *Ascaris lumbricoides* was discovered by Furtado and his colleague in 2019. This marks the first study to prove the presence of polymorphism at codon 200 in *Ascaris lumbricoides* that has been linked with anthelmintic resistance. From a sample size of 854 eggs, 4 eggs (0.5%) were detected with polymorphism at codon 200. Codon 200 of B-tubulin gene has been linked with BZ resistance in STHs, since it was first detected in *Haemonchus contortus*, a helminth with veterinary significance [18]. The occurrence of polymorphism found in codon 200 of B-tubulin gene (0.5%) is much lower than reported studies for other parasites and the author suggested that the

No	Reference	Country	Year
1.	Aissatou D, Drake LJ, Suswillo RR, Kihara J, Bundy DAP, Scott, ME, Halpenny C, Stothard JR, & Prichard RK. (2009). Assays to detect β-tubulin codon 200 polymorphism in *Trichuris trichiura* and *Ascaris lumbricoides*. PLoS Neglected Tropical Diseases, 3(3).	Kenya, Panama, Zanzibar, Uganda	2009
2.	Aïssatou D, Halpenny CM, Churcher, TS, Mwandawiro C, Kihara J., Kaplan RM, Streit TG, Idaghdour Y, Scott ME, Basáñez, MG, & Prichard RK. (2013). Association between Response to Albendazole Treatment and β-Tubulin Genotype Frequencies in Soil-transmitted Helminths. PLoS Neglected Tropical Diseases, 7(5), e2247.	Haiti, Kenya, Panama	2013
3.	Krücken J, Fraundorfer K, Mugisha JC, Ramünke S, Sifft KC, Geus, D, Habarugira F, Ndoli, J, Sendegeya A, Mukampunga, C, Bayingana, C, Aebischer T, Demeler J, Gahutu, JB, Mockenhaupt FP, & von Samson-Himmelstjerna, G. (2017). Reduced efficacy of albendazole against *Ascaris lumbricoides* in Rwandan schoolchildren. International Journal for Parasitology: Drugs and Drug Resistance, 7(3), 262–271.	Rwanda	2017
4.	Zuccherato, LW, Furtado LF, Medeiros C. da S, Pinheiro, C da S, & Rabelo ÉM. (2018). PCR-RFLP screening of polymorphisms associated with benzimidazole resistance in *Necator americanus* and *Ascaris lumbricoides* from different geographical regions in Brazil. PLOS Neglected Tropical Diseases, 12(9), e0006766.	Brazil	2018
5.	Matamoros, Rueda, Rodríguez, Gabrie, Canales, Fontecha, & Sanchez. (2019). High Endemicity of Soil-Transmitted Helminths in a Population Frequently Exposed to Albendazole but No Evidence of Antiparasitic Resistance. Tropical Medicine and Infectious Disease, 4(2), 73.	Honduras	2019
6.	Furtado LFV, Medeiros C. da S., Zuccherato LW, Alves WP, de Oliveira VNGM, da Silva VJ, Miranda GS, Fujiwara RT, & Rabelo, ÉML. (2019). First identification of the benzimidazole resistance-associated F200Y SNP in the beta-tubulin gene in *Ascaris lumbricoides*. PLOS ONE, 14(10), e0224108.	Brazil	2019
7.	Palma A, Matamoros G, Escobar D, Sánchez AL, & Fontecha G. (2020). Absence of mutations associated with resistance to benzimidazole in the beta-tubulin gene of *Ascaris suum*. In Revista da Sociedade Brasileira de Medicina Tropical (Vol. 53). Sociedade Brasileira de Medicina Tropical. https://doi.org/10.1590/0037-8682-0155-2019	Honduras	2020

Table 1: List of publications included in the review.

References	Age Group	Sample Size		Specimens Genotyped	Anthelmintic Distributed
Diawara et al., 2009	School Children	Kenya: Unspecified		38	Albendazole
		Panama: 29 children		29	Pyrantel
		Zanzibar & Uganda: 353 children		91	
Diawara et al., 2013	Adults & Children	Haiti	Pre-treatment: 72	37	Albendazole
			Post-treatment: 11	5	
		Kenya	Pre-treatment: 26	22	
			Post-treatment: 23	19	
		Panama	Pre-treatment:123	53	
			Post-treatment: 70	70	
Krücken et al., 2017	Children (6-10 y/o)	850 children		144	Albendazole
Zuccherato et al., 2018	Adults & Children	110 individuals from 6 states		601	Albendazole
Furtado et al., 2019	Adults & Children	68 individuals from 7 states		854	Albendazole
Matamoros et al., 2019	Children	8 children		452	Day 1; Piperazine Day 2; Albendazole Day 3; Albendazole Day 4; Albendazole
Palma et al., 2020	Domestic Pigs	17 pigs slaughtered for commercial purposes		50	None

Table 2: The age group of study participants, study sample size, number of samples genotyped and anthelmintic used in the reviewed studies.

Reference	Molecular Technique Used	Presence of SNPs	Codon Detected
Diawara et al., 2009	Nested PCR	No polymorphism detected	-
Diawara et al., 2013	Conventional PCR	Polymorphism detected	Codon 167
Krücken et al., 2017	Conventional PCR	No polymorphism detected	-
Zuccherato et al., 2018	PCR-RFLP	No polymorphism detected	-
Furtado et al., 2019	ARMS-PCR	Polymorphism detected	Codon 200
Matamoros et al., 2019	Conventional PCR Used to screen codon 167	No polymorphism detected	-
	Semi-nested PCR Used to screen codon 198 and codon 200	No polymorphism detected	-
Palma et al., 2020	Conventional PCR	No polymorphism detected	-

Table 3: List of the molecular method used to find SNPs in codons 167, 198, and 200 as well as the presence of SNPs and the codon that it was found on.

presence of polymorphism in this study does not points to the primary mode of action of BZ resistance in this species. The author pointed out the possibility that this finding does not indicate a current issue of BZ resistance against *Ascaris lumbricoides*. However, it is a stipulation of a future issue that can increase the prevalence of *Ascaris lumbricoides* in the population, as frequent treatment and re-infection of this species can regulate its progress to develop BZ resistance, especially in rural, tropical areas. Suggestion that there may be other genetic mutations that is causing the BZ resistance, possibly SNPs in different positions in B-tubulin was brought to light in accordance to the low frequency of polymorphisms on codon 167,198 and 200 of β-tubulin gene in *Ascaris lumbricoides*. Theories of a non-drug related mechanisms that corresponds to the resistance seen in *Ascaris lumbricoides* were also suspected as seen in a previous publication of other parasites that showed a different drug-resistance mechanism [4].

From the results, majority of the studies that was reviewed had their participants with a history of anthelmintic consumption through MDA at some point prior to the studies. These studied population has participated in the MDA programmes at least once, while others received yearly or bi-annual MDA. Inconsistent use of anthelmintic drugs allows organisms to adapt and eventually develop resistance to the treatment. The two studies that was able to detect SNPs on codons of β-tubulin gene in Ascaris lumbricoides has a study population that has a history of anthelmintic consumption. This finding suggests that due to the inconsistency of the treatment given to the population, *Ascaris lumbricoides* managed to adapt and mutate its genetic component to become resistant towards anthelmintic. However, due to the limited studies that has been done to prove the correlation of anthelmintic resistance and presence of SNPs at the β-tubulin gene of *Ascaris lumbricoides*, the resistant mechanism of this helminth cannot be confirmed as of the time of this review and this association can only be theorized until further investigations are conducted.

The lack of study done on anthelmintic resistance related markers on *Ascaris lumbricoides* is one of the reasons why there aren't many discoveries of polymorphism on these codons. Previous studies focused more on the ERR and CR rate of the helminths in determining the population's resistance towards anthelmintic rather than considering the molecular approach. The analysis of ERR and CR rate estimates the efficacy of anthelmintic in treating STHs in a population. ERR expresses the reduction of number of eggs after treatment with anthelmintics compared to prior treatment. Meanwhile, CR refers to the participants that are cured after the given treatment. The formula used for ERR and CR is as follows:

$$ERR = \left(\frac{(Mean\ egg\ count\ before\ treatment) - (Mean\ egg\ count\ after\ treatment)}{Mean\ egg\ count\ before\ treatment}\right) \times 100\%$$

Figure 4: Formula for calculating the Egg Reduction Rate (ERR), representing the percentage decrease in mean egg count after treatment.

$$CR = \left(\frac{Number\ of\ patients\ cured\ after\ treatment}{Number\ of\ patients\ infected\ before\ treatment}\right) \times 100\%$$

Figure 5: Formula of Cure Rate (CR), to calculate the percentage of participants cured after treatment.

A high percentage (>80%) of ERR and CR shows that the treatment against STHs were successful and the anthelmintic used is highly efficacious towards the STHs. A percentage lower than 80% might suggest a few causes to the low efficacy of the drug, such as low drug quality, improper treatment or development of resistance [19]. This method does detect the potency of the drug towards the targeted STHs however, it does not prove the presence of anthelmintic resistance, only suggesting a possibility. ERR and CR results should be paired with molecular investigations of SNPs at the β-tubulin genes to confirm the presence of polymorphism causing the STHs resistance towards anthelmintic.

Study contradictions

Similar studies on different helminths have detected

The presence of mutation on codon 167, 198 and 200 of the β-tubulin gene. However, in the reviewed studies, only codon 167 and codon 200 showed polymorphisms that can be related to anthelmintic resistance. The presence of polymorphism observed in *Ascaris lumbricoides* was also significantly lower than that reported in other helminths [18]. Despite the low prevalence of SNPs found in codons that are linked to, it is a stipulation of future emergence of *Ascaris lumbricoides* clusters that would be resistant to anthelmintics should the issue be disregarded.

Conclusion

This review on the presence of anthelmintic resistant markers in *Ascaris lumbricoides* has found that only codon 167 and codon 200 of β-tubulin gene have been detected with the presence of SNPs in relation to anthelmintic resistance. The gene that is associated with anthelmintic resistance can only be detected by molecular investigation. Conventional PCR and a modification of ARMS-PCR are the only method that has been able to detect these SNPs. With the discovery of these SNPs in codons linked to anthelmintic resistance, it is likely that reduced efficacy of anthelmintic towards *Ascaris lumbricoides* may be influenced by these resistance markers.

Author Contributions

M.N. conceptualized the study idea and provided overall supervision. Next, N.M.S. designed the study framework, curation of data, interpretation of the results and prepared the initial manuscript. Both authors reviewed and approved the final version of the manuscript.

Conflict of Interest

No conflict of interest

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