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Molecular detection of benzimidazole resistance levels associated with F167Y and F200Y polymorphisms in *Haemonchus contortus* of goats from Mozambique

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Abstract

Benzimidazole (BZ) resistance of *Haemonchus contortus* has been associated with single nucleotide polymorphisms (SNPs) in codons 200 (F200Y) and 167 (F167Y) and, to a lesser extent, in codon E198A, of the β-tubulin isotype 1 gene. The present study was undertaken to survey the status of BZ resistance in naturally infected goats in smallholder farms in southern Mozambique by real-time PCR (qPCR) using TaqMan® assays. *H. contortus*-infective larvae (L3; $n = 432$) from 12 populations were individually genotyped for F200Y and F167Y SNPs to detect BZ resistance. For the F200Y SNP, the results revealed an overall mean percentages of 18.8% homozygous resistant (RR), 47.8% homozygous susceptible (SS) and 33.4% heterozygous (RS) *H. contortus*. For the F167Y SNP, the overall mean percentages were 1.6% RR, 94.9% SS and 3.5% RS. The percentage of resistant alleles (%R) for the F200Y and F167Y SNPs was 35.7 and 3.4%, respectively. Genotype combinations of the two mutations indicate resistant percentages ranging from 0.0 to 52.9%. From the four herds with high RR individuals, three farms dewormed the animals monthly, while the fourth farm dewormed the animals every 3 months. In farms where animals were dewormed every 6 months, low percentages of RR individuals were found, whereas no RR individuals were discovered in herds where animals were dewormed annually. These results suggest that the F200Y SNP is more significant in BZ resistance development of the surveyed population compared with the F167Y SNP.

Keywords Anthelmintic resistance · β-Tubulin · Gastrointestinal nematodes · Real-time PCR · Small ruminants

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Introduction

Among gastrointestinal nematode (GIN) parasites of major importance in small ruminant production, *Haemonchus contortus* (*H. contortus*) has been identified in many regions worldwide (Akkari et al. 2013; Shija et al. 2014; Mohanraj et al. 2016; Singh et al. 2017a) as a highly pathogenic and economically important parasite (Chaudhry et al. 2015).

Anthelmintic drugs have been commonly used in controlling GIN infections in small ruminants to minimise worm populations and their effects (Waller 2006; McArthur and Reinemeyer 2014). Among the main anthelmintics used are benzimidazoles (BZs), whose frequent and indiscriminate usage because of practicality of administration and reduced cost has culminated in inadequate drug dosing, leading to reduction of efficacy, selection of drug-resistant individuals and a widespread development of anthelmintic resistance (Nunes et al. 2013; Adediran and Uwalaka 2015; Chandra et al. 2015; Mohanraj et al. 2016).

Benzimidazole drugs have been used to control livestock parasites for over 40 years, and resistance to this drug class is at an advanced stage in many parts of the world. The effectiveness of the drugs, therefore, is threatened by the increasing resistance to these compounds in global *H. contortus* populations (Leathwick and Besier 2014). *H. contortus* is one of the most economically important helminth parasites of small ruminants worldwide and is an important model for anthelmintic resistance research (Gilleard 2013; Chaudhry et al. 2016). This parasite has developed resistance to all the major anthelmintic drug classes, and resistance to multiple drug classes occurs, often at high frequency, in many parts of the world (McKellar and Jackson 2004). The increase in the number of resistant individuals in a population is the result of changes in genotypic frequency caused by the reproduction of individuals that have survived drug exposure (Melo and Bevilaqua 2005).

The prevalence and severity of anthelmintic resistance constitute some of the major threats to sustainable productivity of small ruminants worldwide (Waller 2006; Singh et al. 2017b). Although limited, previous studies provided evidence that *H. contortus* is the most prevalent GIN species and that anthelmintic resistance in goat GIN is a significant problem in Mozambique. To date, however, resistance to BZ anthelmintics has been observed by means of in vivo assays only, such as the faecal egg count reduction test (FECRT) in goats (Atanásio et al. 2002; Atanásio-Nhacumbe et al. 2017). Although FECRT is considered the method of choice, it has some limitations owing to low sensitivity, high cost, and laboriousness (Kotze et al. 2012; Peña-Espinoza et al. 2014).

The BZ mode of action blocks the polymerisation of microtubules (Rüfener et al. 2009), inhibiting cell division of parasites by binding to tubulin monomers and leading to microtubule instability (Mohanraj et al. 2016). BZ resistance in *H. contortus* is primarily linked to mutations in the β-tubulin gene that substitutes phenylalanine to tyrosine (TTC/TAC) at codons 167 and 200 and, to a lesser extent, with codon 198 mutations that substitute glutamate by alanine (GAA/GCA), and these substitutions lead to the loss of binding sites for benzimidazoles leading to resistance (Tiwari et al. 2006; Ghisi et al. 2007; Kotze et al. 2012). Molecular techniques that detect the presence of β-tubulin gene mutations conferring BZ resistance are rapid, highly sensitive, and specific in detecting polymorphisms (Tiwari et al. 2006; Ghisi et al. 2007; Nunes et al. 2013).

Early detection of BZ resistance is vital for monitoring and controlling its spread; thus, real-time PCR offers a reliable method for rapid detection and determination of resistance allele frequencies in *H. contortus* polymorphisms (Walsh et al. 2007). The screening of samples from a few animals has the potential to provide information about the benzimidazole resistance status of the entire herd, which would enable a considerable reduction in the costs of diagnosis for the producer. Moreover, molecular diagnosis has practical advantages, since it can guide the choice of anthelmintic drug that will be used, before its application in the herd, thus reducing the economic losses driven by anthelmintic resistance (Nunes et al. 2013).

Therefore, the objective of this study was to determine BZ resistance levels associated with F167Y and F200Y polymorphisms in the β-tubulin isotype 1 gene of *H. contortus* in field populations of goats from southern Mozambique by real-time PCR (qPCR).

Materials and methods

Location and study animals

The study was carried out between November 2016 and December 2016 in 12 goat farms in seven districts of the southern region of Mozambique, namely Xai-Xai (XA; $n = 2$), Chibuto (CH; $n = 3$) and Chókwe (CK; $n = 1$) in the Gaza province and Magude (MA; $n = 1$), Boane (BO; $n = 2$), Namaacha (NA; $n = 1$) and Moamba (MO; $n = 2$) in the Maputo province. The samples were subsequently genotyped between February 2017 and May 2017.

Mozambique is located in the southern hemisphere in Southeast Africa, between the latitudes 10° 28′ S and 26° 52′ S and longitudes 30° 12′ E and 42° 51′ E, with an altitude of 0–2400 m (Gelcer et al. 2017). The study areas were located between 24° 38′ S and 26° 02′ S latitude and 33° 39′ E and 32° 07′ E longitude; they were characterised by a tropical dry climate influenced by the motions of the Indian Ocean, with a hot rainy season from October to March and a cool dry season from April to September (Diniz et al. 2012).

A total of twelve farms, six in the Gaza province and six in the Maputo province were selected. In order to assess the profile of zoo technical management systems, a questionnaire was administered to the owners or managers of the farms as was described by Atanásio-Nhacumbe et al. (2017). The number of goats in each of the flocks varied from 26 to 180 animals, and three flocks, two in Gaza (XA1 and CH1) and one in Maputo (NA1), were kept extensively on communal grazing without supplementation throughout the year, while the rest of the flocks were kept semi-intensively on private delimited pasture areas with supplementation during the dry season. For the study purposes, only 26 to 30 goats were randomly allocated to two groups of 13–15 animals in each one of the flocks; thus, a total of 355 goats were surveyed. The 12 populations of *H. contortus* studied were obtained from goat herds of mixed breeds (Landim, Boer, Kalahari red, and Saanen) and different ages (adults and young of both sexes).

Faecal examination and determining anthelmintic resistance

Faecal samples were collected directly from the rectal bulb of 26 to 30 goats per herd without deworming for at least 90 days.

From the obtained samples, individual eggs per gram (EPG) counts were performed and animals that presented with EPG counts above 200 with more than 40% of trichostrongylid eggs were used for coprocultures (Ueno and Gonçalves 1998; Gupta and Singla 2012) of pooled samples per herd. Third-stage larvae (L3) of GIN used in the study were obtained from coprocultures; each herd constituted a population from which 36 *H. contortus*-infective larvae (L3) were recovered for individual DNA extraction and genotyping of F167Y and F200Y polymorphisms in the β-tubulin gene. Only *H. contortus* was chosen for genotyping since it is the most pathogenic gastrointestinal nematode parasite and whose qPCR assay was standardised and in use in the laboratory where the study was conducted. The efficacy of oral suspensions of albendazole (5 mg/kg body weight; Albenol-100®, Interchemie, Holland), mostly used in Mozambique, was assessed using the FECRT. The latter was performed according to the methods recommended by the World Association for the Advancement of Veterinary Parasitology (Coles et al. 1992; Coles et al. 2006) and interpreted using the RESO® software (CSIRO, 1990 Animal Health Division) to determine anthelmintic resistance as was described by Atanásio-Nhacumbe et al. (2017).

DNA extraction and genotyping of F167Y and F200Y polymorphisms

To determine genotypic and allelic frequencies, a total of 432 *H. contortus* L3 were individually subjected to DNA extraction using a commercial kit according to the manufacturer's instructions (PureLink® Genomic DNA; Invitrogen, Carlsbad, California (CA), USA). Genotyping of F200Y and F167Y polymorphisms in the β-tubulin gene was performed by real-time PCR (7500 Real-Time PCR System; Applied Biosystems, Foster City, CA, USA) and TaqMan® assay. For genotyping of the F200Y SNP, locked nucleic acid (LNA) probes and primers were used as described by Walsh et al. (2007), whereas the probes and primers used for the F167Y SNP were described by Lambert et al. (2017).

PCR was conducted as described by Lambert et al. (2017). End-point analysis was performed in duplicate to classify larvae based on homozygous or heterozygous resistance and susceptibility alleles for the respective mutations using the Genotyping Experiments of the 7500 Real-Time PCR System (Applied Biosystems). The Brazilian isolates S-IVM, São Paulo State University (UNESP), Botucatu campus (Echevarria et al. 1991), and Embrapa 2010 (Embrapa CPPSE; Chagas et al. 2013) were used as susceptible and resistant controls, respectively.

Data analysis

Genotypic and allelic frequencies were calculated for each SNP in each population. Differences in genotypic and allelic frequencies within and between populations as well as between provinces were analysed by chi-square tests using the Statistical Package for Social Science (SPSS; version 21, IBM Software, USA) software, and p values < 0.05 were considered significant. Associations between %FECR and management systems, as well as between genotypes (RR, SR, SS) for F200Y and F167Y SNPs, were analysed by Mann–Whitney test. A correlation analysis between FECRT phenotypes (%FECR), RR, SR and SS genotypes, and S and R phenotypes for F200Y and F167Y SNPs, relating to semi-intensive and extensive management systems was run.

The nematode parasites were considered phenotypically resistant to BZ when one of the following combinations of genotypes was detected (F167Y/F200Y): homozygous susceptible (SS)/homozygous resistant (RR), RR/SS, heterozygous SR/SR, RR/SR, SR/RR or RR/RR as suggested by Barrère et al. (2012, 2013). High resistance was assumed when the sum of the percentage of resistance genotype combinations was greater than 30% (von Samson-Himmelstjerna et al. 2009; Lambert et al. 2017).

Results

Genotyping F200Y and F167Y SNPs in the β-tubulin gene of 432 *H. contortus* L3 originating from 12 goat populations of southern Mozambique confirmed BZ resistance as resistance alleles for F167Y and F200Y SNPs were identified in the DNA samples examined (Fig. 1). This was characterised by the presence of the RR genotype, with amplitudes of inter-population variation of 2.8–52.9% for F200Y SNP and 2.8–5.6% for F167Y SNP (Table 1).

The overall mean frequencies of F200Y SNP genotypes were 47.8% SS, 33.4% SR and 18.8% RR with significant differences among all populations analysed for SS ($p = 0.000$), RR ($p = 0.000$) and SR ($p = 0.000$). The MA1 and MO2 populations of the Maputo province showed the highest frequencies of resistant F200Y SNP genotypes at 52.9 and 47.2%, respectively; the average allelic frequencies for this SNP were 64.3% allele S and 35.7% allele R with inter-population-significant difference for both alleles ($p = 0.000$), taking into account all studied populations. The presence of heterozygous individuals (5.6–94.4%) in nearly all populations strongly contributed to the percentage of resistance alleles observed for the F200Y SNP (2.8–67.6%).

For the F167Y SNP, the mean genotype frequencies were 94.9% SS, 3.5% SR, and 1.6% RR with significant differences among all populations for the SS ($p = 0.000$) and SR ($p = 0.037$) genotypes; no statistical differences were observed for the RR genotype among all populations ($p = 0.513$). The highest percentage of RR for the F167Y SNP was observed in two populations of the Gaza province (CH3 and CK1) and did not exceed 5.6%. The average allelic frequency percentage

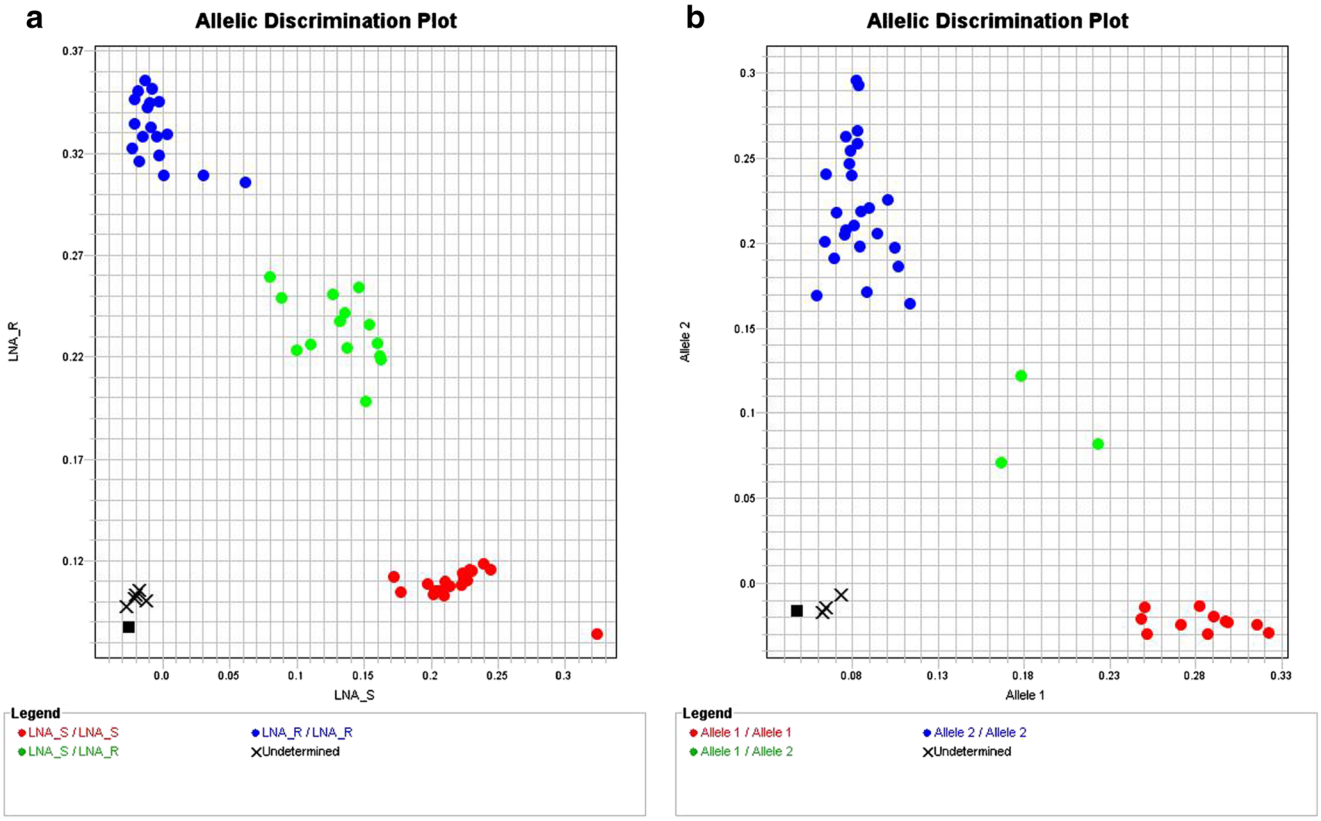

Fig. 1 Plot of allelic discrimination from the qPCR reaction of F200Y (**a**) and F167Y (**b**) SNPs of the β-tubulin gene from individuals of *H. contortus* from goat herds from Mozambique

Table 1 Genotypic and allelic frequencies of F200Y and F167Y polymorphisms in *H. contortus* from 12 populations of goats in Gaza and Maputo provinces, Mozambique

Locations	Population	*N*	Genotypic and allelic frequencies (%)									
			F200Y					F167Y				
			RR*	SR*	SS*	R*	S*	RR	SR*	SS*	R*	S*
Xai-Xai	XA1	36	30.6	36.1	33.3	48.6	51.4	0	0	100	0	100
Xai-Xai	XA2	36	36.1	30.6	33.3	52.8	47.2	0	11.1	88.9	5.6	94.4
Chibuto	CH1	36	0	0	100	0	100	0	5.6	94.4	2.8	97.2
Chibuto	CH2	36	0	94.4	5.6	47.2	52.8	2.8	5.6	91.6	5.6	94.4
Chibuto	CH3	36	0	5.6	94.4	2.8	97.2	5.6	0	94.4	5.6	94.4
Chókwe	CK1	36	19.5	72.2	8.3	55.6	44.4	5.6	2.8	91.6	6.9	93.1
Total Gaza		*216*	*14.4*	*39.8*	*45.8*	*34.5*	*65.5*	*2.3*	*4.2*	*93.5*	*4.4*	*95.6*
Magude	MA1	36	52.9	29.4	17.7	67.6	32.4	0	0	100	0	100
Namaacha	NA1	36	0	13.9	86.1	6.9	93.1	0	0	100	0	100
Boane	BO1	36	16.7	38.9	44.4	36.1	63.9	0	0	100	0	100
Boane	BO2	36	2.8	25	72.2	15.3	84.7	0	0	100	0	100
Moamba	MO1	36	19.4	27.8	52.8	34.7	65.3	2.8	8.3	88.9	6.9	93.1
Moamba	MO2	36	47.2	27.8	25	61.1	38.9	2.8	8.3	88.9	6.9	93.1
Total Maputo		*216*	*23.2*	*27.1*	*49.7*	*36.9*	*63.1*	*0.9*	*2.8*	*96.3*	*2.3*	*97.7*
Overall Mean		*432*	*18.8*	*33.4*	*47.8*	*35.7*	*64.3*	*1.6*	*3.5*	*94.9*	*3.4*	*96.6*

* Allelic and genotypic frequencies with statistically significant differences between inter-populations considering total sample populations ($p < 0.05$)

was 96.6% allele S and 3.4% allele R with inter-population significant difference for both alleles (S, $p = 0.000$; R, $p = 0.001$), taking into account all studied populations (Table 1).

From the four herds with high RR individuals for F200Y SNP, three farms (XA1, 30.6%; XA2, 36.1%; MO2, 52.8%) dewormed the animals monthly, while the fourth farm (MA1, 52.9%) drenched the animals every 3 months. In farms where animals were dewormed every 6 months, low percentages of RR individuals were found (BO2, 2.8%; CH2, 8.4%; BO1, 16.7%; MO1, 22.2%; CK1, 25%), whereas no RR individuals were discovered in herds where animals were dewormed annually (CH1 and CH3) or where levamisole was being used despite the past usage of benzimidazoles (NA1).

For both SNPs, no statistical differences between Gaza and Maputo provinces were observed ($p > 0.05$) regarding the overall mean genotypic frequencies, namely the RR, SR, and SS genotypes, as well as allelic frequencies of R and S. The distribution of the associations between SNPs (F167Y/F200Y) shows that 20.4% of individuals analysed (88/432) possessed associations that generate resistance phenotypes. The homozygous genotype susceptible to the F167Y mutation and the homozygous genotype resistant to the F200Y mutation (F167Y-SS/F200Y-RR) were the most frequent among populations (18.5%), followed by the heterozygous combination of both mutations (F167Y-SR/F200Y-SR = 0.9%) and the F167Y SNP homozygous resistant and F200Y SNP heterozygous genotypes (F167Y-RR/F200Y-SR = 0.7%). Combinations of homozygous resistant individuals in both mutations (F167Y-RR/F200Y-RR) were not observed in this study. Out of all the populations studied, four (33.3%), namely XA1, XA2, MA1, and MO2, exhibited resistance levels higher than 30%; populations MA1 and MO2 had the highest percentages at 52.0 and 52.8%, respectively (Table 2). On the other hand, the majority of the populations were susceptible to BZ and three of which were 100% susceptible (CH1, CH3 and NA1). It is worth noting, however, that the resistance allele R is present in all these populations, as all possess heterozygous individuals for at least one of the SNPs analysed (Table 2). Faecal egg reduction data (%FECR) were added in Table 2 for comparison of genotypic and phenotypic data.

No correlations were observed among the variables analysed, namely FECRT phenotypes (%FECR), RR, SR and SS genotypes, and S and R phenotypes for F200Y and F167Y SNPs, relating to semi-intensive and extensive management systems. No association was observed between %FECR and management systems ($p = 0.164$), but there was an association between resistance phenotype (R) for F200Y SNP relating to management systems ($p = 0.018$).

The analysis of the zoo technical management information as per the questionnaire administered indicated that in all of the flocks (100%), the main objective was to produce goat meat; the animals were kept either in communal or private pastures during day hours and shared the pastures with cattle and/or sheep. The animals were reared under extensive systems in communal pastures during the day and kept in corrals at night in 27.3% of the farms (flocks XA1, CH1 in Gaza and flock NA1 in Maputo), and in the rest of the flocks (72.7%), the goats were reared under semi-intensive systems in private pastures and kept in cement or metal slabs at night.

Table 2 Percentage of susceptible (S) and resistant (R) phenotypes in 12 populations of *H. contortus* obtained from goats of southern Mozambique, based on the combination of F167Y and F200Y SNP genotypes of the β-tubulin gene, and the %FECR (Atanásio-Nhacumbe et al. 2017)

		Populations											
Genotype	Phenotype (%)	XA1	XA2	CH1	CH2	CH3	CK1	MA1	NA1	BO1	BO2	MO1	MO2
167-SS/200-SS	S	33.3	25.0	94.4	5.6	91.6	2.8	17.7	86.1	44.4	72.2	44.4	19.4
167-SS/200-SR	S	36.1	30.6	0	86.0	2.8	69.4	29.4	13.9	38.9	25.0	25.0	22.2
167-SR/200-SS	S	0	8.3	5.6	0	5.6	0	0	0	0	0	8.4	5.6
167-SR/200-SR	R[b]	0	0	0	5.6	0	2.8	0	0	0	0	0	2.8
167-RR/200-SS	R[b]	0	0	0	0	0	5.6	0	0	0	0	0	0
167-SS/200-RR	R[b]	30.6	33.3	0	0	0	19.4	52.9	0	16.7	2.8	19.4	47.2
167-RR/200-SR	R[b]	0	0	0	2.8	0	0	0	0	0	0	2.8	2.8
167-SR/200-RR	R[b]	0	2.8	0	0	0	0	0	0	0	0	0	0
167-SR/200-RR	R[b]	0	0	0	0	0	0	0	0	0	0	0	0
	% S	*69.4*	*63.9*	*100*	*91.6*	*100*	*75.0*	*47.1*	*100*	*83.3*	*97.2*	*77.8*	*47.2*
	% R	*30.6*[a]	*36.1*[a]	*0*	*8.4*	*0*	*25.0*	*52.9*[a]	*0*	*16.7*	*2.8*	*22.2*	*52.8*[a]
	%FECR	*96*	*0*[c]	*100*	*34*[c]	–	*0*[c]	*68*[c]	*81*[c]	*97*	*51*[c]	*68*[c]	*72*[c]

[a] Percentage of resistance phenotype > 30%

[b] Combinations classified as resistant (F167Y/F200Y) were SS/RR, RR/SS, SR/SR, RR/SR, SR/RR or RR/RR (Barrère et al. 2012)

[c] %FECR < 95 is resistance phenotype using FECRT (Coles et al. 1992)

The animals were reared under extensive systems in communal pastures during the day and kept in corrals at night in 36.4% of the farms, whereas for the rest of the herds (63.6%), goats were reared under semi-intensive systems in private pastures, kept in slabs at night, and were grazed together with sheep and/or cattle. In 75% (9/12) of the farms, BZ was used to drench animals based on visual estimative weight; however, in farms CH2 and CK1, BZ was replaced by ivermectin and moxidectin because of supposed failure in efficacy, while levamisole was used in farm NA1. All animals were dewormed with anthelmintics on each occasion, and no counts of eggs per gram (EPG) of faeces tests were performed prior to deworming in all farms. Anthelmintics were chosen without any criteria, and no quarantine was undertaken in case of acquiring animals from other countries or from other regions within the country in all surveyed herds. The data from the questionnaire indicated also that most of the farms have poor management practices, including inadequate disease control measures and inadequate nutrition during the dry season, and face disease challenges, mainly gastro-intestinal parasitism.

Discussion

Using molecular techniques, studies identifying resistance levels to BZ in natural populations of *H. contortus* in Mozambique are scarce. Although we expected resistance levels to be high—because of the breeding and management systems of these locations—it was found that the resistance levels are not yet of great concern regarding the mutations present in the F167Y and F200Y SNPs evaluated. To the best of our knowledge, this is the first survey to report the profile of genotypic and allelic frequencies of these two SNPs in *H. contortus* populations of Mozambique.

As evidenced by the qPCR results, different levels of BZ resistance in *H. contortus* mainly associated with the F200Y polymorphism were observed in other studies undertaken in Eastern Canada, Europe, India, and Pakistan which suggested that this SNP is widespread and often highly frequent, whereas changes in allele frequencies at codon 167 are relatively low (Barrère et al. 2013) or did not occur (Tiwari et al. 2006; Chaudhry et al. 2015; Chaudhry et al. 2016).

High prevalence of the F200Y polymorphism was reported in Pakistan (Chaudhry et al. 2016) and India (Tiwari et al. 2006; Chaudhry et al. 2015), while no polymorphisms at codon 167 were found in both countries. Tiwari et al. (2006) reported higher percentages of RR genotypes in three regions surveyed for F200Y SNP presence in India and that overall prevalence of the BZ-resistant allele was 86%, indicating the importance of F200Y SNPs in determining BZ resistance profiles of *H. contortus* in the studied populations of India and Pakistan.

In another BZ resistance survey of *Haemonchus*, *Teladorsagia* and *Trichostrongylus* species carried out in Ireland, Italy, and Switzerland, Ramünke et al. (2016) reported that in all populations studied, the mutation at codon 200 was the most common SNP under selection, even at different frequencies among countries where farms presented with BZ resistance-associated SNP frequencies above 10%; however, F167Y and E198A SNPs were rarely observed.

In contrast, a study conducted by Lambert et al. (2017) on goats from the Bahia State of Northeast Brazil reported that the percentage of larvae RR for the F200Y SNP was relatively low (18.9%) when compared with F167Y (32.7%). The authors also observed that the associations between these two SNPs indicated percentages of resistance ranging from 34.7 to 100% between populations. Moreover, the highest percentages of RR were found for codon 167, indicating that the F167Y SNP is relatively more prevalent and relevant than F200Y in these regions of Brazil, whereas the opposite was observed in Mozambique. The differences observed in resistance genotype and allele frequencies among studies from several countries—apart from differences in management production systems—could possibly be attributed to the differential prevalence of *H. contortus* strains in each country, as this nematode shows enormous genetic diversity. Moreover, the differences in nematode response to anthelmintic treatments are also associated with the genetic diversity of the parasite (Gilleard and Beech 2007; Papadopoulos 2008).

The percentages of individuals with the resistant genotype RR (30.6–52.9%) were higher in only four (33.3%) of the populations surveyed in this study. When analysing the genotype combination of the two SNPs, we observed that double-heterozygous individuals—which confer a resistant phenotype according to Barrère et al. (2012)—were present at low frequencies yet contributed to the resistance profile in three of the studied populations (CH2, CK1 and MO2).

Using qPCR, the frequency of the resistant genotype of *H. contortus* was detected as 30.6% in the XA1 herd. In the same herd, FECRT detected a percentage faecal egg count reduction (%FECR) of 96% using the method recommended by Coles et al. (1992), where more *Trichostrongylus* spp. (59%) were detected than *Haemonchus* spp. (37%) as reported by Atanásio-Nhacumbe et al. (2017). The same pattern was found in herd BO1 where the %FECR was 97% and more *Strongyloides* spp. (79%) were detected than *Haemonchus* spp. (19%), according to the same study; however, qPCR assays detected a resistant genotype frequency of 16.7%. Esteban-Ballesteros et al. (2017) suggested that because FECRT cannot detect resistance levels less than 25%—especially under field conditions where GIN infections are typically of mixed species composition—determining the frequency of resistant alleles in pools or in individual L3 can be an alternative to FECRT, as FECRT only represents an estimate of the resistance in a herd naturally infected with nematode

parasites when the prevalence of anthelmintic resistance is relatively high.

The frequency of BZ usage and the practice of deworming entire herds in some of the farms surveyed may have contributed to the frequencies of RR individuals observed in this study. Previous studies have reported that inappropriate use of anthelmintics has contributed to their efficacy failure, leading to the widespread development of anthelmintic resistance in GIN parasites of small ruminants (Adediran and Uwalaka 2015; Chandra et al. 2015; Salgado and Santos 2016).

Although there are favourable conditions for the development of BZ resistance in the herds studied here, our data only showed moderate levels of resistance; taking into account F200Y SNP and the low frequencies of F167Y SNP, both add up to nearly 20.4%-resistant homozygous individuals. This drew our attention to the possibility of E198A SNP influence, which was not evaluated in this study and may be determining resistance levels in the region. The fact that many SS individuals for both F167Y and F200Y SNPs were detected does not necessarily mean that they are all susceptible to BZs; however, it reveals the possibility that these individuals may possess a mutation in codon 198, making them resistant to the drug. It is worth mentioning that the present study investigated the F200Y and F167Y mutations based on their significant role in the development of BZ resistance in *H. contortus* of goats in some countries (Chaudhry et al. 2015; Chaudhry et al. 2016; Lambert et al. 2017).

There is a discrepancy in the data obtained between molecular tests and in vivo tests performed on these same herds by Atanásio-Nhacumbe et al. (2017), as this study demonstrated the occurrence of very low frequencies of RR individuals and high frequencies of SR individuals for the F200Y polymorphism in some herds, while %FECR and the presence of *H. contortus* larvae that survived the post-treatment coprocultures indicated high levels of resistance to albendazole (%FECR < 95) according to Coles et al. (1992), namely in herds CH2, CK1, BO2, MO1 and NA1, where no RR individuals were detected. Apart from the farms XA1 and CH1, where no *H. contortus* larvae were found in post-treatment coprocultures, the high resistance phenotypic data using the FECRT could be an indication of existence of resistance of *H. contortus* to albendazole than of other nematode species since only 1% of *Oesophagostomum* spp., 6% of *Trichostrongylus* spp. and 9% of *Strongyloides papillosus* were present in post-treatment coprocultures against 84% of *H. contortus* in farm XA2, 50% of *Oesophagostomum* spp. and 50% of *H. contortus* in farm MA1, while high numbers of *H. contortus* (100%) were found in the rest of the farms. The low frequency levels of RR genotypes of F200Y SN may be evidence of E198A SNP presence. Although E198A has not been investigated in this study, it has been identified to play a role in the development of BZ resistance in South Africa (Ghisi et al. 2007), a neighbouring country of Mozambique and from where Boer and Kalahari Red goats in sampled herds in this study where acquired by Mozambican farmers.

In resistant isolates of *H. contortus* from Australia, Kotze et al. (2012) reported that while individuals could be heterozygous at codons 200 and 198, the homozygosity at 198 in the highly resistant individuals is mutually exclusive with heterozygosity or resistant homozygosity at codon 200. Their study also indicated that the presence of E198A SNP in *H. contortus* larvae is associated with a higher level of thiabendazole resistance than F200Y SNP presence. Zhang et al. (2016) corroborated the findings of Kotze et al. (2012); in a study of eight *H. contortus* populations from China, they reported that the frequencies of resistant E198A SNP were 0–70 and 0–31% for resistant F200Y SNP, demonstrating higher frequencies of E198A SNP compared with the F200Y SNP, whereas the F167Y SNP was not detected. These findings reinforce the possibility of a role for the E198A SNP in the development of BZ resistance in some *H. contortus* populations surveyed in southern Mozambique; this is evidenced by the low %FECR levels (< 95%), which shows albendazole inefficacy against GIN, including *H. contortus*, in goat populations of Mozambique.

Conclusion

The occurrence of F200Y and F167Y polymorphisms in the gene encoding β-tubulin protein was demonstrated in *H. contortus* field populations from goats of southern Mozambique. In general, low levels of BZ resistance associated with F200Y SNP were detected; however, the emergence of resistance associated with F167Y SNP was also observed. The study revealed low frequencies of RR genotype individuals of both SNPs; however, moderate to high frequencies of heterozygous individuals were observed. The presence of resistant alleles associated with the type of reproduction of the parasites and the disordered use of anthelmintics may contribute to the development of resistance in these populations. Therefore, suitable measures for monitoring and controlling anthelmintic resistance must be implemented to reverse the current resistance status and to prevent further spread of BZ resistance development in the studied region.

The present study provides evidence for the higher significance of F200Y SNP than F167Y in the development of BZ resistance and the need for further studies to determine the possible association between E198A SNP and BZ resistance in *H. contortus* from goats of southern Mozambique once FECRT revealed high phenotypic resistance levels. Furthermore, our results indicate a risk of the progressive spread of BZ resistance in *H. contortus* if current technical management practices are maintained.

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Compliance with ethical standards

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. Approval from the Ethical Commission on the Use of Animals at the School of Veterinary Medicine—Federal University of Bahia (UFBA), Brazil, has been registered under EMZV-UFBA No. 09/2017.

Conflict of interest The authors declare that they have no conflict of interest.

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