Veterinary Parasitology 199 (2014) 160–164

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Identification and quantification of benzimidazole resistance polymorphisms in *Haemonchus contortus* isolated in Northeastern Brazil

Jessica Maria Leite dos Santos[a], Jomar Patrício Monteiro[b], Wesley Lyeverton Correia Ribeiro[a], Iara Tersia Freitas Macedo[a], Ana Lourdes Fernandes Camurça-Vasconcelos[a], Luiz da Silva Vieira[b], Claudia Maria Leal Bevilaqua[a,*]

[a] *Programa de Pós-graduação em Ciências Veterinárias/Universidade Estadual do Ceará, Faculdade de Veterinária, Universidade Estadual do Ceará, Av. Dedé Brasil, 1700, CEP 60714-903 Fortaleza, CE, Brazil*
[b] *Empresa Brasileira de Pesquisa Agropecuária – Caprinos e Ovinos, Estrada Sobral/Groaíras, km 04, Caixa Postal 145, CEP 62010-970 Sobral, CE, Brazil*

ARTICLE INFO

Article history:
Received 24 June 2013
Received in revised form 1 November 2013
Accepted 9 November 2013

Keywords:
Haemonchus contortus
Quantitative real-time PCR
β-Tubulin gene
SNP
Sheep

ABSTRACT

Haemonchus contortus is the most prevalent nematode in Brazil. The objective of this study was to select 6 populations of *H. contortus* of known or suspected benzimidazole resistance status and characterize these using quantitative real-time polymerase chain reaction (qPCR) for single nucleotide polymorphisms (SNPs) F200Y, F167Y and E198A in the β-tubulin isotype 1 gene. qPCR was performed using DNA from a pool of 10 adult male *H. contortus* from a single animal per farm. Faecal egg count reduction test (FECRT) and egg hatch test (EHT) were used to determine the resistance status. Samples were obtained from 6 farms located in 5 counties in the Ceará State: Tauá, Boa Viagem, Quixadá, Santa Quitéria and Solonópole. The inbred-susceptible-Edinburgh (ISE) isolate was used as reference for comparative purposes in the qPCR. Benzimidazole resistance was detected by FECRT on all farms with efficacy values ranging from 0 to 51%. EC50 values as determined by EHT were all above 1.49 μg/ml. High frequencies of the resistant SNPs F200Y and F167Y alleles were detected but no resistance was detected at SNP E198A. Our results suggest that the SNPs F167Y and F200Y are both important for benzimidazole resistance in the studied populations.
© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Haemonchus contortus is the most prevalent nematode infecting sheep in Brazil (Veríssimo et al., 2012). Parasitism is controlled with the use of anthelmintics which may lead to selection of resistant nematodes within populations (Falzon et al., 2013).

Benzimidazoles (BZs) are an anthelmintic class adopted worldwide in small ruminant nematode control and drug resistance is a global problem (Alvarez-Sánchez et al., 2005; Falzon et al., 2013). This resistance is known to be associated with SNPs F200Y, F167Y and E198A in the β-tubulin isotype 1 gene (Ghisi et al., 2007). Quantitative real-time PCR (qPCR) provides a viable alternative to traditional methods, allowing rapid identification and quantification of resistant and susceptible alleles in pools of nematodes of the same species which in turn may create

* Corresponding author at: Programa de Pós-graduação em Ciências Veterinárias/FAVET/UECE, Av. Dedé Brasil, 1700, Campus do Itaperi, CEP 60714-903 Fortaleza, Ceará, Brazil. Tel.: +55 85 31019853; fax: +55 85 31019840.
E-mail addresses: claudia.bevilaqua@pq.cnpq.br, bevilaqua.uece@gmail.com, claudiamlb@yahoo.com.br (C.M.L. Bevilaqua).

0304-4017/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetpar.2013.11.006

a reasonable window for changes in strategies for parasite control (Walsh et al., 2007; Molento et al., 2011).

The objective of this study was to characterize BZ resistance through qPCR in BZ resistant *H. contortus* isolates from different locations in the Ceará State, Northeast Brazil.

2. Materials and methods

2.1. Resistant and susceptible isolates

BZ resistant isolates were obtained from 6 farms located in 5 counties of Ceará State, Northeast Brazil: Tauá (TA1 and TA2), Boa Viagem (BV), Quixadá (QX), Santa Quitéria (SQ) and Solonópole (SO). Faecal egg count reduction test (FECRT) and egg hatch test (EHT) were used to determine the resistance status of the populations. The BZ susceptible inbred-susceptible-Edinburgh (ISE) isolate was used as reference of susceptibility in the qPCR (Roos et al., 2004; Walsh et al., 2007).

2.2. The faecal egg count reduction test

FECRT were performed on a minimum of 10 animals per farm. The sheep were treated with 5 mg/kg body weight of oxfendazole orally. Faecal samples were obtained as previously described, on day zero and 8 days after treatment (Coles et al., 1992). Eggs per gram (epg) were determined using the modified McMaster technique (Ueno and Gonçalves, 1998). Faecal samples were cultured to obtain L3 and a minimum of 100 L3 were identified.

2.3. Egg hatch test

Nematode eggs were collected as described by Hubert and Kerboeuf (1992). EHT was performed according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles et al., 1992).

2.4. Collection of BZ resistant adult male H. contortus

Experimental sheep were treated with oxfendazole prior to euthanasia in order to select BZ resistant parasites. Adult male nematodes were collected, identified as described by Ueno and Gonçalves (1998) and preserved in 80% ethanol at −20 °C. The methodology used was approved by the Ceará State University Ethics Committee (protocol number: 11585428-2/04).

2.5. Quantitative real-time PCR

Genomic DNA was extracted from pools of 10 adult male *H. contortus* per farm. Pools were macerated in liquid nitrogen and incubated overnight at 56 °C in 500 μl digestion buffer (0.2% SDS, 50 mM EDTA, 50 mM Tris-HCl, 0.4 mg/ml proteinase K, 100 μg/ml RNase, pH 8.0). SDS was removed by incubation on ice in the presence of potassium acetate and DNA was alcohol precipitated. DNA was further subjected to two rounds of phenol:chloroform (1:1) purification prior to further alcohol precipitation. The final pellet was resuspended in TE buffer and stored at −20 °C.

Table 1
Real time PCR primers used to detect *H. contortus* SNPs F200Y; F167Y and E198A. In the primer name column, F stands for forward and R for reverse.

Primer	Sequence 5′–3′	Allele
200R2	CAGAGCTTCGTTGTCAATACAGA	Sensitive
200R1	CAGAGCTTCGTTGTCAATACAGT	Resistant
200F2	CTACCCTTTCCGTCCATCAA	Both
167F1	CCTGATAGAATTATGGCTTCGTT	Sensitive
167F2	CCTGATAGAATTATGGCTTCGTA	Resistant
167R1	GATCTCACCTTGGGTGATGG	Both
198R1	CTTCGTTGTCAATACAGAATGTTT	Sensitive
198R2	CTTCGTTGTCAATACAGAATGTTG	Resistant
198F1	GAAGATGTTTTAAGGTATCCGACACT	Both

Primers for all three SNPs were designed considering a general annealing temperature of 58 °C using the Primer3 Plus program (Untergasser et al., 2007) based on sequences obtained from GenBank (accession numbers: GQ910877.1 to GQ910916.1) (Table 1). β-tubulin isotypes 1 and 2 sequences were checked during primer design in order to make primers as specific to isotype 1 as possible and avoid cross amplification of isotype 2 β-tubulin gene.

Quantitative PCR assays were performed with DNA extracted from one pool per farm and in triplicate for each pool. Reactions contained 12.5 μl 2× Fast Start Universal SYBR Green Master Mix (Roche, West Sussex, UK), 0.3 pmol/μl of each primer (forward and reverse) (Table 1), 25 ng of DNA and water for a total volume of 25 μl. Negative controls used water instead of DNA. Amplification conditions for SNPs F200Y and F167Y were: 95 °C for 10 min and 35 cycles at 95 °C for 15 s and at 58 °C for 30 s. SNP E198A amplification used only 34 cycles. Melting curve analysis was applied to detect primer dimers. Amplified products were visualized on a 3% agarose gel after electrophoresis to check for nonspecific amplicons.

2.6. Data analysis

Oxfendazole efficacy was determined based on epg data before and after treatment using the software BootStreat (INRA, version 1.0) (Kochapakdee et al., 1995). BZ resistance was also characterized using the EHT to determine effective concentrations that inhibit 50% of larvae hatching (EC50). Populations were considered resistant in the FECRT and EHT as per the WAAVP recommendations (Coles et al., 1992).

The threshold cycle (Ct) for each qPCR reaction was determined by the software Realplex 2.2 (Eppendorf) and allele frequencies were estimated using a previously described formula (Germer et al., 2000). In cases where it was not possible to determine a Ct value (e.g., no amplification detected) for a given allele, its frequency was set to zero and the frequency of its detected counterpart was approximated to 100%.

3. Results

Based on FECRT, BZ resistance was present in all studied farms. Individual epg values and FECRT efficacy percentages are described in Table 2. *Haemonchus* spp. was the most predominant in all farms after anthelmintic treatment (Table 3). *Trichostrongylus* spp. and *Oesophagostomum*

Download English Version:

https://daneshyari.com/en/article/5803822

Download Persian Version:

https://daneshyari.com/article/5803822

Daneshyari.com