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Abstract

Coccidiosis of sheep and goats is caused by protozoa in the genus *Eimeria*. These protozoa mainly a f ect young animals, causing a decrease in production and consequent economic losses. Routine diagnosis is made through morphological observation of the oocysts, which has several limitations. The objective of the present study was to develop a real-time PCR (qPCR) technique for the diagnosis of *Eimeria spp.* in sheep and goats. For this purpose, the 18S rRNA region of the DNA of these parasites was selected because it is a region with low variability among *Eimeria spp.* The qPCR technique was developed using SYBR Green, resulting in a PCR with high sensitivity, and the ability to amplify samples containing only one oocyst of an *Eimeria spp.* There was no cross-reaction

repeatability test showed that the coe f cient of variation was less than 2%. This indicated that this method has good

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premierbioso .com/primerdesign). e primers obtained were named *Eimeria* 18S-F (5 -CAGGCTTGTCGCCCTGA-3) and *Eimeria* 18S-R (5 -TTCGCAGTAGTTCGTCTTT-3), with an amplicon of 168 bp.

DNA extraction steps were performed in accordance with product instructions of the Stool DNA Kit (E.Z.N.A Stool DNA Kit, OMEGA, Bozeman, MT, USA).

Endpoint PCR was used with primer sets to obtain the PCR product of *Eimeria spp.* e reaction mixture (25 μL) contained 2.5 μL PCR bu er (10x), 2.0 μL dNTPs (2 mM each), 1.0 μL MgCl^2 (25 mM), 1.0 μL forward and reverse primers (10 μM), e PCR .009 (f)]TJ/TT1 1 (er)6(s)0 0

measured in triplicate and plotted against the logarithm of their initial copy number. Each standard curve was generated by linear regression of the plotted points, and standard curve parameters were obtained. Ct was calculated under default settings for the qPCR so system So ware (ver. 4.0, Analytik).

Each assay designed included negative and positive controls and the standard curve. e negative control was the PCR reaction without template DNA. e positive control was a PCR reaction containing DNA of *Eimeria spp.* All controls and samples were assayed at least three times. e speci city of *Eimeria spp.* qPCR was evaluated by

C. parvum simultaneously. e analytical sensitivity (i.e., limit of detection) was established using eight replicates of serially diluted pMD18T-168 plasmid at 3.43×10^7, 10^6, 10^5, 10^4, 10^3, 10^2, 10^1, and 100 copies/reaction. gDNA was extracted from 10,000 coccidia oocysts, and diluted into ve gradients of 10,000, 1,000, 100, 10, and 1 oocyst(s) according to the tenfold dilution series. e established qPCR was used to detect gDNA to determine the sensitivity of the method. e interassay precision of the qPCR was de ned as the coe cient of variation (CV) of Ct values obtained for each copy number/reaction in three di erent assays performed on 3 di erent days.

e developed qPCR method and McMaster method were used to monitor the *Eimeria spp.* infection quantity of four naturally infected lambs for 4 weeks and to compare the di erence between two methods [19-21].

Data are presented as mean (± SD). e pMD18T-168 DNA levels

was used for statistical analysis.

e PCR ampli cation product of *Eimeria spp.* 18S rRNA was detected by 1% agarose gel electrophoresis, and the ampli ed fragment was between 100 bp and 250 bp, which encompassed the expected fragment size (168 bp). e sequencing results of positive clones were compared with sequences (MW512853.1) in GenBank, with a matching rate of 100% (Figure 1 and Table 1).

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