SHORT COMMUNICATION

https://doi.org/10.21708/avb.2022.16.4.10988

The *Rv2807* target gene: a determining factor to directly detect *Mycobacterium bovis* from suspected bovine tuberculosis lesions

Gene alvo Rv2807*: um fator determinante para detectar* Mycobacterium bovis *diretamente de lesões suspeitas de tuberculose bovina*

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ABSTRACT: Bovine tuberculosis (bTB) is a zoonosis caused by *Mycobacterium bovis*, a species belonging to the *Mycobacterium tuberculosis* complex (MTC) group. Direct bTB diagnosis from suggestive lesions can be performed by *nested* q-PCR targeting the *Rv2807* gene present in the MTC group, as well as the *TbD1* gene, present in *M. bovis*. In this context, the aim of the present study was to assess the importance of considering positive MTC results for the *Rv2807* target gene obtained through the nested real time polymerase chain reaction (*nested* q-PCR) applied to samples obtained directly from suspected bTB lesions. A total of 174 samples of suggestive bTB caseous lesions were obtained during cattle slaughter in slaughterhouses in the state of Mato Grosso, Brazil. DNA was extracted from the lesions and *nested* q-PCR was performed to detect both MTC and *M. bovis*. Both samples positive for the *Rv2807* (41/174) and *TbD1* (29/174) were submitted to bacterial culturing (23/41), and the DNA of the isolates (23) was extracted and submitted again to *nested* q-PCR. The *Rv2807* gene (MTC) was previously amplified by *nested* q-PCR directly from the lesions, although the *TbD1* gene specific for *M. bovis* was not amplified previously in four of the successfully isolated samples (4/23), only following isolation, and only the *Rv2807* gene was amplified before and after isolation. In conclusion, the target gene *Rv2807* (MTC) exhibited higher positivity in the analyzed samples compared to the *TbD1* gene (*M. bovis*).

KEYWORDS: *Nested* real time PCR, molecular diagnosis, *Mycobacterium tuberculosis* complex, Zoonosis.

RESUMO: A tuberculose bovina (bTB) é uma zoonose causada pelo *Mycobacterium bovis*, uma espécie pertencente ao grupo do complexo *Mycobacterium tuberculosis* (MTC). O diagnóstico direto de bTB a partir de lesões sugestivas pode ser realizado por nested q-PCR visando o gene *Rv2807* presente no grupo MTC, bem como o gene *TbD1*, presente em *M. bovis*. Nesse contexto, o objetivo do presente estudo foi avaliar a importância de considerar os resultados de MTC positivos para o gene alvo *Rv2807* obtidos através da reação em cadeia da polimerase nested real time (nested q-PCR) aplicada a amostras obtidas diretamente de lesões suspeitas de bTB. Um total de 174 amostras de lesões caseosas sugestivas de bTB foram obtidas durante o abate de bovinos em frigoríficos do estado de Mato Grosso, Brasil. DNA foi extraído das lesões e nested q-PCR foi realizado para detectar tanto MTC quanto *M. bovis*. Ambas as amostras positivas para *Rv2807* (41/174) e *TbD1* (29/174) foram submetidas a cultura bacteriana (23/41), e o DNA dos isolados (23) foi extraído e submetido novamente à nested q-PCR. O gene *Rv2807* (MTC) foi previamente amplificado por nested q-PCR diretamente das lesões, embora o gene *TbD1* específico para *M. bovis* não tenha sido amplificado anteriormente em quatro das amostras isoladas com sucesso (4/23), apenas após o isolamento, e apenas o gene *Rv2807* foi amplificado antes e após o isolamento. Em conclusão, o gene alvo *Rv2807* (MTC) apresentou maior positividade nas amostras analisadas em relação ao gene *TbD1* (*M. bovis*).

PALAVRAS-CHAVE: *Nested* PCR em tempo real, diagnóstico molecular, *Mycobacterium bovis*, Zoonose.

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Received: 02/18/2022. Accepted: 05/24/2022

INTRODUCTION

M. bovis is the cause of bovine tuberculosis (bTB) in several mammals, including humans, and is a neglected zoonosis comprising a significant public health problem, with the particularity of being clinically identical to human tuberculosis caused by *Mycobacterium tuberculosis* (CARVALHO et al., 2016; SÁNCHEZ-CARVAJAL et al., 2021). *M. bovis* belongs to a group of tuberculosis-causing mycobacteria, identified as the *Mycobacterium tuberculosis* complex (MTC). Members of this group exhibit nucleotide sequence similarity above 99% (GAGNEUX, 2018).

PCR assays are alternatives used in the rapid diagnosis of bTB, as they are able to detect *M. bovis* in fragments of tuberculosis-suggestive lesions, increasing detection sensitivity and specificity and decreasing the diagnosis timeframe (FURLANETO et al., 2012b; CARVALHO et al., 2015). A bTB diagnosis is confirmed by the detection of the *Rv2807* genes present in MTC species, according to Araújo et al. (2014b), as well as the *TbD1* gene present in *M. bovis* (including BCG strains), as well as in *M. africanum*, *M. canettii*, which are not present in modern *M. tuberculosis* strains (PEREIRA et al., 2017; MA et al., 2022).

In this context, the aim of the present study was to assess the importance of considering positive MTC results for the *Rv2807* target gene obtained through the *nested* q-PCR applied to samples obtained from suspected bTB lesions.

MATERIAL AND METHODS

A total of 174 suspected bTB caseous lesion samples were obtained through partial or total condemnation of bovine carcasses during slaughter in slaughterhouses in the state of Mato Grosso (Brazil), through sanitary inspections carried out by the State Sanitary Inspection Service (SISE) and the Federal Inspection Service (SIF). The samples were submitted to DNA extraction and then analyzed by *nested* q-PCR to detect MTC through the target *Rv2807* gene and *M. bovis* by detecting the *TbD1* gene, according to Araújo et al. (2014a, 2014b), modified by Carvalho et al. (2015). All samples positive for *TbD1* (*M. bovis*) and *Rv2807* (MTC) were submitted to microbiological culturing, first undergoing a decontamination process using 0.75% hexadecylpyridinium chloride (HPC) and 10% sulfuric acid (H_2SO_4) (AMBRÓSIO et al., 2008; FURLANETTO et al., 2012b) and then seeded in duplicate in tubes containing Stonebrink's medium and incubated at 37°C for 90 days (COUSINS et al., 1989; BRAZIL, 2008; OIE, 2018). Following microbial growth, DNA was extracted from the obtained isolates according to Van Soolingen et al. (1991) and confirmed through amplification of the *TbD1* gene specific for *M. bovis* through *nested* q-PCR.

RESULTS AND DISCUSSION

Of the 174 samples of lesions suggestive of bTB analyzed by *nested* q-PCR, 23.56% (41/174) tested positive for the *Rv2807* gene (MTC), and of these, 70.73% (29/41) were positive for the *TbD1* gene (*M. bovis*), confirming the presence of *M. bovis* in the analyzed tissue samples. Among the 41 (41/174) MTC positive and 29 (29/41) *M. bovis* positive samples, bacillus viability was verified by bacterial isolation in Stonebrink medium for 56.09% (23/41) of the samples. Of these, four (4/23) tested positive for MTC by *nested* q-PCR previously in the first q-PCR performed directly from the deficiencies. After isolation, the *Rv2807* and *TbD1* genes ware confirmed in all 23 cultured samples by *nested* q-PCR (Table 1). These findings demonstrate the importance of considering samples positive for bTB even if only for the MTC specific *Rv2807* gene has been amplified, corroborating previous assessments by Araújo et al. (2014a), while the target gene *Rv2807* (MTC) exhibited greater positivity in the analyzed samples in relation to the *TbD1* gene (*M. bovis*).

Furthermore, although microbiological cultivation is considered the "gold standard" technique (SÁNCHEZ-CARVAJAL et al., 2021), this methodology should not be used individually for the identification and definitive confirmation of bTB, as 43.90% (18/41) of the 41 positive did not exhibit any microbial growth. This corroborates the fact that *M. bovis* is difficult to isolate, while also indicating the presence of mycobacteria in bTB-suspected caseous lesions, the inadequacy of the isolation method through bacilli inactivation during the sample decontamination process, multiplication difficulty during microbiological culturing and bacillus inactivation caused by the organism's immune defense and/or microbial competition with other microorganisms present in the sample (FRÁGUAS et al., 2014; KANIPE; PALMER, 2020).

Nested q-PCR is an alternative for bTB diagnosis, as the method is easy to install inside slaughterhouses and can be validated by the National Program for the Control and Eradication of Brucellosis and Tuberculosis (PNCETB). The use of *Rv2807* gene *nested* q-PCR would aid in controlling and eradicating the disease more effectively, as a quicker detection of positive bTB animal and identification of the source of the disease focus would result in quicker bTB sanitation in affected areas and, consequently, bTB control and eradication. The state of Mato Grosso (Brazil) is one of the world's largest beef cattle producers (SEMAGRO, 2017), displaying a low prevalence of bTB (FURLANETTO et al., 2012a; NÉSPOLI et al., 2016), and would thus benefit from the application of a molecular technique for bTB diagnosis, as, when moving towards a pathogen eradication process, there is a need for faster, more sensitive and specific confirmation techniques (CARVALHO et al., 2015). In this regard, bTB control and eradication is paramount, as this is a zoonotic disease that causes significant economic losses to producers, states and the country (BRAZIL, 2006).

The efficiency of the *nested* q-PCR technique in detecting MTC directly from bovine tissues has been previously described by Carvalho et al. (2015), surpassing even PCR-Multiplex and microbiological culture tests. However, factors related to DNA amount and dilution or the number of viable bacilli in the investigated samples can interfere with the results,

Table 1. *Nested* q-PCR (suspected bTB lesions), microbiological culture and *nested* q-PCR (isolates) of tissue samples from suspected bTB caseous lesions detected in bovine carcasses during slaughter in slaughterhouses in the state of Mato Grosso, Brazil, from 2018 to 2019.

Samples	Results			
	nested q-PCR from tissue lesion		Microbiological Culture	*nested* q-PCR
	Rv2807 gene MTC	*TbD1* gene *M. bovis*		*TbD1* gene *M. bovis*
1	+	+	+	+
2	+	+	+	+
4	+	+	+	+
5	+	+	+	+
8	+	+	+	+
24	+	+	+	+
33	+	+	-	/
35	+	+	+	+
41	+	-	+	+
43	+	+	+	+
45	+	+	-	/
56	+	+	+	+
60	+	+	-	/
69	+	+	-	/
71	+	-	-	/
82	+	-	-	/
86	+	+	-	/
90	+	-	-	/
101	+	-	-	/
103	+	-	-	/
106	+	+	-	/
109	+	+	-	/
110	+	-	-	/
113	+	+	+	+
114	+	-	-	/
116	+	+	+	+
118	+	+	+	+
119	+	+	+	+
120	+	-	-	/
121	+	+	+	+
122	+	+	+	+
123	+	+	+	+
124	+	-	+	+
128	+	-	+	+
130	+	-	+	+
133	+	+	+	+
134	+	+	+	+
135	+	+	+	+
154	+	+	-	/
160	+	+	-	/
173	+	+	-	/
Total	41 (41/174)	29 (29/41)	23 (23/41)	23 (23/23)
%	23.56%	70.73%	56.09%	100%

/ = not performed; % = percentage

as they directly affect the sensitivity of PCR systems concerning *M. bovis* diagnosis (PEREIRA et al., 2017).

According to Furlanetto et al. (2012b), high amounts of bacilli in suspicious caseous (paucibacillary) lesions following recent bTB infections are not common. This may, therefore, compromise the diagnosis.

The four samples from suspicious lesions that tested negative in *nested* q-PCR for the *TbD1* gene (*M. bovis*) and positive following culturing are mostly from young animals (three animals from 2 to 3 years old and one over 3 years old). This corroborates the results reported by Furlanetto et al. (2012b) and reinforces the hypothesis that these four tuberculosis cases detected by *nested* q-PCR following culturing were most likely isolated from animals exhibiting recent infections.

CONCLUSION

In sum, positive results for *Rv2807* gene (MTC) *nested* q-PCR results should not be ruled out during the investigation of *M. bovis* from DNA directly extracted from suspected bTB lesions, as the *Rv2807* gene detects species belonging to the *M. tuberculosis* complex, in addition to being more sensitive than the detection of specific mycobacteria from the MTC complex). The target gene *Rv2807* (MTC) displayed greater positivity in the analyzed samples compared to the *TbD1* gene (*M. bovis*).

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