019

EVALUATION OF PCR METHODS FOR THE MOLECULAR DETECTION OF BABESIA CABALLI AND THEILERIA EQUI ON FIELD SAMPLES

Bartolomé Del Pino L.E. [2], Cersini A.[1], Scicluna M.T.[1], Nardini R.[1], Manna G.[1], Antognetti V.[1], Autorino G.L.[1]

[1]National Reference Centre for Equine Diseases (CERME), Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana ~ Rome ~ Italy, [2]Complutense University of Madrid, Madrid, Spain; National Reference Centre for Equine Diseases (CERME), Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana ~ Rome ~ Italy

Keywords: Equine piroplasmosis, Diagnosis, PCR

INTRODUCTION:

Equine piroplasmosis, a life threatening tick-borne disease caused by B. caballi and T. equi, is subject to international movement restrictions. While antibodies can be lifelong, as also for the carrier state, the OIE prescribed tests are still serologically based, even if these may be negative at the beginning of infection [5]. Also, the definition of the carrier state, employing direct methods is especially important in endemic areas for justifying and verifying the treatment efficacy due to its potential toxicity. For this, a study was conducted for the adoption of molecular methods for the diagnosis of these infections by evaluating the performance of different PCR methods, traditional and Real Time (RT), on field samples for both types of infections.

MATERIALS AND METHODS:

103 whole blood samples of clinically suspect equids, collected within a research project of the CERME, were analysed using 4 different PCRs for each protozoan. Genomic DNA was extracted using Cador Pathogen 96 QIAcube HT Kit (Qiagen®). PCR protocols were conducted as described in literature or according to instructions. For T. equi (T): end point PCR (T1) and nested-PCR (T2), targeting equine merozoite antigen (EMA) complex gene (amplicons 268bp and 102bp respectively) [1,6]; RT PCR (T3) targeting the 18S gene (81bp) [4]; Path-T. equi Genesig ® (T4) targeting EMA 1 (about 120bp). For B. caballi (B): End point PCR (B1) and nested PCR (B2), targeting rhoptry associated protein complex gene (825bp and 430bp respectively) [1,3]; RT PCR (B3) targeting the 18S gene (95bp) [2]; Path-B. caballi (Genesig ®) (B4) targeting the 18s gene (about 100bp). The specificity of discordant results was verified by sequencing. The PCR detecting the greatest number of positives was chosen for assessing relative sensitivity (rSe) and relative specificity (rSp). Agreement among the PCRs was estimated for each protozoan.

RESULTS:

Number of positives per method are as follows: for B1 (4); B2 (8); B3 (4); B4 (2); for T1 (29); T2 (29); T3 (35); T4 (27). An overall agreement of 91.3% was observed for B1 and 90.3% for T1. Table 1 reports the number of samples in agreement for 2, 3 and 4 PCRs. As B3 and T3 detected the highest number of positive samples and the discordant were specific products, they were used as reference tests to estimate the rSe and rSp, reported in Table 2.

	Number of PCRs in agreement					
	Babesia Caballi			Theileria equi		
	4	3	2	4	3	2
Positive	1	0	5	26	1	4
Negative	93	4		67	5	

Table 1: number of samples in agreement for 2, 3 and 4 PCRs for B. Caballi and T. equi

	T3	
	rSe	rSp
T1	80.00	98.53
T2	82.86	100.00
T4	77.14	100.00

	B3	
	rSe	rSp
B1	25.00	96.97
B2	50.00	93.94
B4	50.00	100.00

Table 2: rSe and rSp of PCRs against reference test

DISCUSSION AND CONCLUSIONS:

B3 and T3 were considered the best PCRs probably due to their primer efficiency and their short amplicons. Moreover, T3 primers were designed within a highly conserved region, and B3 employs an MGB probe consenting the use of shorter targets. The lower rSe of B1 and B2 could be due to the higher mutation frequency or degradation of their long targets, but recruitment of a major number of positives is necessary to verify this result. In general, a good overall agreement (>90%) for the PCRs of each parasite is observed. The major positivity in PCR for T. equi could be due to its reported marked higher parasitemia and prevalence than that of B. caballi [2,5]. Furthermore, from this preliminary study, when compared to the serological tests, the PCRs identified carriers among the seronegatives, as well as non-carriers among the seropositives. For T. equi, of the 36 PCR positive, 17 were seronegative and for B. caballi, all PCR positives were seronegative. In view of these results, B3 and T3 can be employed in routine diagnosis and developed as quantitative methods to assess correlation between parasitemia and the clinical phase of infection to aid the clinician, in deciding or verifying treatment. Moreover, it would be recommendable for international movement control to include PCR, in adjunct to sero-methods in use.

REFERENCES:

1) Battsetseg B. et al.; Int J Parasitol. 2001; 31(4):384-6. 2) Bhoora R. et al.; Vet Parasitol. 2010;168(3-4):201-11. 3) Bhoora R. et al.;Vet Parasitol. 2010;169(3-4):279-88. 4) Kim C. et al.; Vet Parasitol. 2008;151(2-4):158-63. 5) Mehlhorn H., Schein E.;Parasitol Res. 1998;84(6):467-75. 6) Nicolaiewsky T.B. et al.; Vet Parasitol. 2001;31;101(1):9-21.