047 Addition of PCR methods to conventional serology for the routine diagnosis of equine piroplasmosis

Maria Teresa Scicluna, *Roberto Nardi, Leticia Elisa Bartolomé del Pino, Ida Ricci, Francesca Rosone, Gian Luca*

Istituto Zooprofillatico Sperimentale delle Regioni Lazio e Toscana, Rome, Italy (Scicluna teresa.scicluna@izslt.it ; Nardini, Bartolomé del Pino, Ricci, Rosone, Autorino)

Equine piroplasmosis is a tick-borne protozoal infection generally difficult to diagnose clinically as clinical signs are non-specific. The infection also represents an important constraint to the international movement of equids. Common practice is the sole use of serologic tests for piroplasmosis, and for those available there is limited data on their validation. Reliable laboratory tests or their combination are essential. In a previous comparative study, real-time (RT) PCRs targeting the rRNA 18s gene (1,2), 1 for each parasite, were adopted on the basis of their optimal performance. This study presents and discusses their use in conjunction with the OIE-prescribed serologic tests: ELISA (VMRD, USA) and indirect immunofluorescence (IFAT, Fuller Laboratories, USA) to analyze blood samples from 274 horses. For comparison of the results, the PCR was used as the reference method since sequencing of derived amplicons confirmed these as *T. equi* and *B. caballi*. Case definition for an acute form of piroplasmosis was temperature >38°C and at least one of the following signs, jaundice, anemia, and petechial hemorrhages, and a PCR positive (+) result.

The *B. caballi* RT-PCR detected 14 + samples, none confirmed in ELISA, while the IFAT had a sensitivity (Se) of 50% (7/14) and a specificity (Sp) of 87.7 % (228/260). The *T. equi* RT-PCR detected 137 + samples with the ELISA showing a Se of 67.2% (92/137) and a Sp of 83.2% (114/137) and the IFAT, a Se of 86.1% (118/137) and a Sp of 81% (111/137). For both parasites, the IFAT, even if still limited, presented an apparently major sensitivity when compared to the ELISA, but liable to cross-reactivity. Results for *B. caballi* are preliminary, due to the limited number of + samples recruited.

Of the PCR + horses for *B. caballi* and *T. equi*, only 28.7% and 19.7% respectively were defined as cases, possibly due to the persistence of parasites beyond the acute form. In particular, for T. equi the serological tests showed a high agreement and a relatively high specificity. In this circumstance, the PCR negative samples could be due to the sterilization of the infected horses, occurring spontaneously or following treatment. With the further introduction of quantitative PCR, treatment efficacy could be monitored especially in view of the side effects these possess. **This study demonstrates that the simultaneous use of PCR with serologic tests increases the diagnostic probability to define the sanitary state for equine piroplasmosis for the purposes stated above.**

References

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