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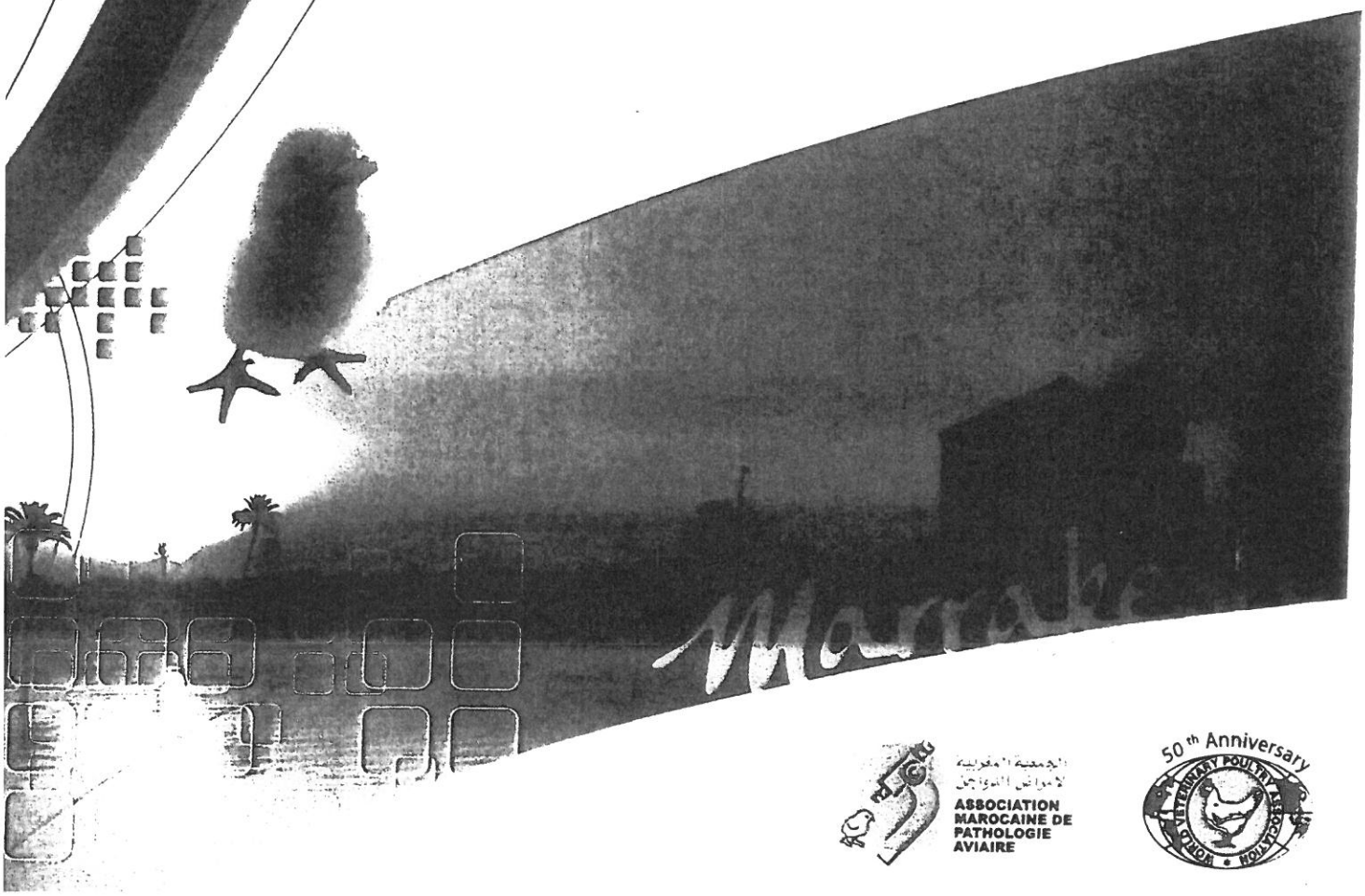

ASSOCIATION MAROCAINE DE PATHOLOGIE AVIAIRE

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Detection of *Enterococcus faecalis* ST82 associated with amyloid arthropathy by real-time PCR

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Amyloid arthropathy (Aa) has often been observed in brown layers or other types of layers and less frequently in broiler parents. It may be accompanied by systemic amyloidosis, involving mainly the liver and spleen. The infection is often unnoticed until the condition has turned chronic and the flock has become uneven with important economic consequences (Petersen et al. 2008). *Enterococcus faecalis* is considered to play a significant role in the pathogenesis (Landman et al. 1998; Petersen et al. 2008). We have previously demonstrated that a specific clone, ST82, as determined by multilocus sequence typing (MLST), is associated with Aa, and that it has a world-wide distribution (Petersen et al. 2009). However, sequencing of seven housekeeping genes is time consuming and expensive, adding to the costs associated with outbreaks. The aim of this study therefore was to develop a Real-Time (RT)-PCR method for rapid and specific detection of the lineage of E. faecalis responsible for amyloid arthropathy in layers, allowing proper actions to be taken as early as possible to improve animal welfare and decrease losses associade with Aa. Single-nucleotide polymorphisms of ST82 were analysed using the software Minimum SNP. Primers were subsequently designed with the software Primer3 using allele sequences as template. RT-PCR was run with Maxima SYBR Green qPCR Master Mix (Fermentas) on a MxPro3000 (Stratagene). The primers were first tested against three isolates with known base-pairs at the specific sites. Subsequently 12 isolates were selected from our collection and RT-PCR was performed in a blind test. After the SNPs were resolved, a full MLST sequence type was determined for the 12 isolates. MLST was performed on the selected isolates according to the standard protocol. Minimum SNP detected two ST82 single-nucleotide polymorphisms that in combination made up an unambiguous identification of the sequence type in question. The test allowed a 100% identification of ST82 among alleles known in the E. faecalis MLST database. The new method allows a rapid screening of E. faecalis isolated from suspected cases of Aa in layers with results available after few hours from primary blood agar plates. However, in heavy breeds it will not be specific for amyloid arthropathy since we have recently found ST82 to be involved in other lesions in this type of poultry (Bisgaard et al. 2009).