Establishment of the first PCR for the identification of *Brachyspira alvinipulli*

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INTRODUCTION

Avian intestinal spirochetosis (AIS) is a common enteric disease of poultry caused by colonisation of the large intestine with anaerobic spirochetes of the species *Brachyspira*. *B. pilosicoli, B. intermedia* and *B. alvinipulli* are currently considered to be pathogenic for poultry. Other *Brachyspira* spp. are non-pathogenic but may be found in mixed infections. Laboratory diagnostics of *Brachyspira* species is essential for confirming clinical diagnosis, providing data for optimal treatment and for surveillance of the bacteria in individual animals or herds.

The aim of this study was to extend a previously published diagnostic multiplex-PCR (1) **for further differentiation of avian *Brachyspira* species and specifically for the identification of *B. alvinipulli.***

METHODS

1. NCBI GenBank was screened for a *B. alvinipulli* specific gene using BLAST search.
2. Oligonucleotide primers putatively specific for *B. alvinipulli* tlyA gene encoding a putative hemolysin were designed according to general primer design guidelines using the PrimerQuest tool (https://eu.idtdna.com/PrimerQuest/Home/About) in coordination with the previously published multiplex primers (1).
3. Samples used were 16 isolated *B. alvinipulli* strains as well as reference strains of the following species: *B. suanatina, B. hampsonii, B. murdochii, B. innocens, B. hyodysenteriae, B. pilosicoli, B. intermedia.*
4. DNA was extracted from all isolates using the DNeasy Kit (Qiagen, Hilden, Germany) according to the manufacturer.
5. PCR was first run as a singleplex followed by sequencing of the product to confirm identity and specificity. Finally, the multiplex setup including the new *tly*A primers was run. PCR conditions were identical to the previously published method (1).

CONCLUSIONS

Our study describes the first *Brachyspira alvinipulli* specific PCR and a novel diagnostic multiplex-PCR suitable for differentiation of the aetiological agents of AIS, including *B. alvinipulli.*

RESULTS

1**.** Based on in silico analysis, the hemolysin A gene (*tly*A ; accession: KF386106) was chosen for putative identification of *B. alvinipulli.* All 12 *B. alvinipulli* isolates showed the expected 160 bp amplicon (Fig.2). Sequencing confirmed the identity and thus, the specific amplification of the *tly*A gene (Fig.1)

2.The *B. alvinipulli tly*A primers were then integrated into the original multiplex PCR setup. Screening of all 12 isolates with this novel multiplex PCR resulted in the 160 bp amplification product of the *tly*A gene. Seven *Brachyspira* reference strains were tested in the original as well as in the novel multiplex PCR including *tly*A primers. Results showed that the integrated strains of other *Brachyspira* species did not generate the 160 bp *tly*A amplicon (Fig.3)**.**

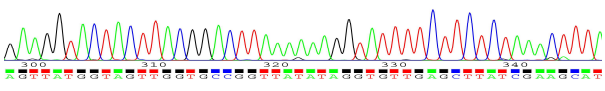

Fig. 1: Sequencing result confirming the identity of the 160 bp amplicon

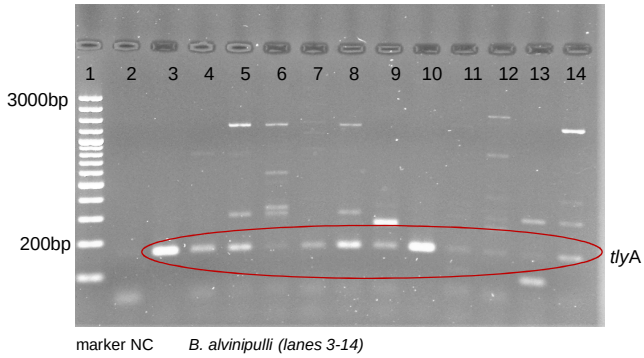

Fig. 2 : Agarose gel electrophoresis (1.5%) showing the 160 bp PCR product amplified from *B. alvinipulli* using the new *tly*A primers in a singleplex PCR

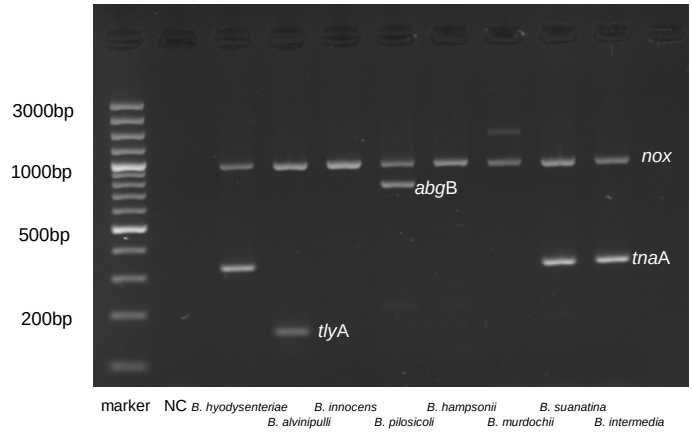

Fig. 3 : Agarose gel electrophoresis (1.5%) showing the species-specific PCR products amplified from several *Brachyspira* spp. using the extended multiplex primer sets

References
(1) Harms, M., Schmidt, V., Heydel, T., Hauptmann, J., Ahlers, C., Bergmann, R. & Baums, C. (2018) Differentiation of *Brachyspira* spp. isolated from laying hens using PCR-based methods and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Vet Diagn Invest. 30(4):545-553.