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Short communication

Molecular analysis of *Trichomonas gallinae* in racing pigeons from Upper Silesia, Poland

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Abstract

The aim of the study was the molecular analysis of ITS1/5.8S rRNA/ITS2 region of *Trichomonas gallinae* isolates from racing pigeon lofts in Upper Silesia, Poland. The analysed region is very useful for the taxonomy of the Trichomonadidae family and indicates the possible existence of different genotypes or species within the *T. gallinae*. A comparison of the complete ITS1-5.8S-ITS2 region of obtained sequences revealed two different sequences. Twenty-three of the isolates (62%) showed the first sequence (KU954106) while fourteen isolates (38%) showed the second sequence type (KU954107), which were homologous with sequences from Genbank. The phylogenetic analysis showed that the two *T. gallinae* genotypes which occurred in the pigeons from Upper Silesia are widespread among European countries.

Key words: *Trichomonas gallinae*, racing pigeons, Poland, ITS1/5.8S rRNA/ITS2

Introduction

Avian trichomonosis is a parasitic disease caused by the flagellated protozoan *Trichomonas gallinae*. The natural host is thought to be Columbidae which has been considered responsible for the worldwide spread of *T. gallinae* among other birds – passerine and psittacine birds as well as falconiformes (Grabensteiner 2010). The parasite is commonly found in the upper digestive tract and the pathologic changes associated with infection range from mild inflammation of the mucosa to large caseous lesions. Studies carried out on the gene 5.8S rRNA and the surrounding Internal Transcribed Spacer regions (ITS) have proved to be very useful for the taxonomy of the Trichomonadidae family, and molecular techniques have indicated the possible existence of different genotypes or species within the *T. gallinae* morphologic complex, which might vary in different areas (Felleisen 1997, Kleina et al. 2004, Gerhold et al. 2008). The aim of the study was the molecular analysis of *T. gallinae* isolates from racing pigeon lofts from Upper Silesia, Poland.

Materials and Methods

The oral swabs were taken from the oral cavity and crop of 100 racing pigeons during the veterinarian examination. The birds come from the Upper Silesia region which is the major base for racing pigeons in Poland. The swabs which were positive during microscopic examination were placed in 5 ml of Medium 199 with Earle salts, 15% foetal calf serum and 22 mg

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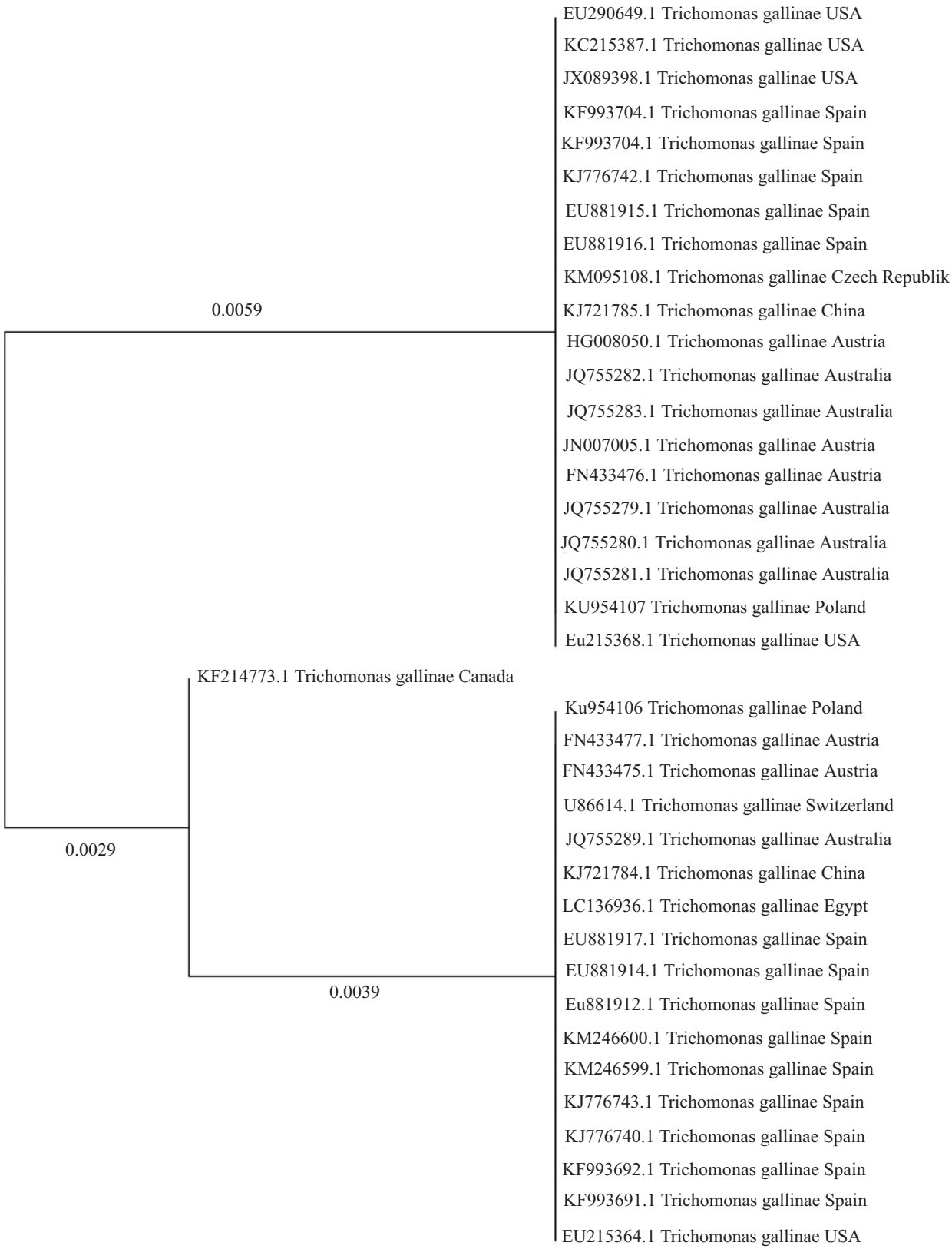

Fig. 1. Phylogenetic tree of *T.gallinae* isolates from different countries based on the complete ITS1-5.8S-ITS2 gene region.

rice starch, and incubated for 24 hours at 37ºC for parasite multiplication. DNA was obtained from the sediment after medium centrifugation (400g x 5 min) and by using a commercial DNA extraction kit (A&A Biotechnology) in accordance with the manufacturer's instructions. PCR reactions were carried out following the method used by Felleisen (1997) with TFR 1 (5'-TGCTTCAGCTCAGCGGGTCTTCC3') and TFR 2 (5'-CGGTAGGTGAACCTGCCGTTGG-3') oligonucleotides. Amplification products were electrophoresed in a 1.5% agarose gel, stained with Midori Green, and visualized under UV light (Quantity One Biorad). PCR products of 350bp size were excised from the gel and purified using the Gel-out extraction kit (A&A Biotechnology) according to the manufacturer's instructions, and sent for sequencing to Macrogen Europe. The ITS1-5.8S rRNA-ITS2 region sequences were analyzed using Mega 5 and the obtained data were compared with GenBank sequences.

Results and Discussion

A total of 37 trichomonad DNA positive samples were found out of 100 racing pigeons which results in a 37% infection level, which is similar to other pigeon populations in Europe (Catelli et al. 1999, Lennon et al. 2013). A comparison of the complete ITS1-5.8S-ITS2 region of all the obtained sequences revealed two different sequence types among examined strains. Twenty three isolates (62%) showed the first sequence type which has been deposited in the EMBL database under accession number KU954106. The remaining fourteen isolates (38%) showed the second sequence type which has been deposited under accession number KU954107. Those two sequence types were homologous with sequences previously placed in Genbank (Fig. 1). Those two genotypes are widely spread in European countries such as Spain, Switzerland Austria and Czech Republic, neighbouring Poland. Sansano-Maestre et al. (2009) who investigated the ITS1-5.8S-ITS2 region of *T. gallinae* obtained from domestic pigeons and birds of prey named those two genotypes A and B. They noticed that genotype A was more prevalent in columbiformes, which is supported by our results. Genotype B was more often found in raptors as well as in all birds that displayed macroscopic lesions. In our study there was no visible typical trichomonosis lesions in beak cavity of any of the examined birds which suggest that there is no connection between the genotype and patogenicity. We are not able to say that all birds carrying the *T. gallinae* genotype B were free of lesions, because the genetic material of *T. gallinae* came from the culture taken from the swabs, obtained during the vet examination, without necropsy. Our analysis shows that the two *Trichomonas* genotypes which occur in the Upper Silesia region are widespread among European countries.

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