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Short Note

Trichinella spiralis in road-killed raccoon dogs (*Nyctereutes procyonoides*) in western Poland

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ABSTRACT. Trichinellosis is still one of the most important food-borne parasitic zoonoses and is considered as a threat to public health worldwide. The aim of this study was to use genotyping techniques to determine the prevalence of *Trichinella* species in wild raccoon dogs (*Nyctereutes procyonoides*) in western Poland. The infection rate in raccoon dogs was 0.8%. All infections were due to *T. spiralis*.

Key words: *Trichinella spiralis*, *Nyctereutes procyonoides*, raccoon dogs, Poland, PCR

Introduction

Trichinellosis, a disease caused by roundworms belong to the genus *Trichinella*, is still one of the most important food-borne worldwide parasitic zoonoses. To date, epidemiological studies have shown sympatric occurrence of four species of *Trichinella* (*Trichinella spiralis*, *T. nativa*, *T. britovi* and *T. pseudospiralis*) in Poland [1–4]. They are characterized by natural (sylvatic) and domestic (synantrophic) environment circulation cycles and by the different hosts species. Transmission between hosts can only occur through the ingestion of meat with infective stages of the parasite. Trichinellosis is considered as a threat to public health worldwide [5]. In Poland, *Trichinella* spp. have been found to be prevalent in domestic and wild animals, most of which were carnivores and omnivores in Poland [6–8].

The aim of this study was to determine the prevalence of *Trichinella* species in the population of wild, road-killed raccoon dogs (J) (*Nyctereutes procyonoides*) in western Poland and to use molecular tools to identify the *Trichinella* species that had been recovered.

Materials and Methods

In 2012, muscle samples were collected at irregular intervals from carcasses of 39 raccoon dogs that had been killed accidentally on roads in western Poland in Warta Mouth National Park between the towns Kostrzyń and Słońsk (n=20) and in the Forestry Bogdaniec (n=19) near to the towns Bogdaniec (n=6), Motylewo (n=4), Marwice (n=5) and Jasiniec (n=4) (Fig. 1).

The muscle samples were taken from the diaphragm. We isolated *Trichinella* muscle larvae (ML) by digesting about 200g of tissue in a HCL-pepsin solution [9]. Digested samples were washed in H_2O and any larvae (ML) present were counted and the number of larvae per gram of muscle tissue (LPG) was calculated. Larvae were stored in 75% ethyl alcohol until genotyping. Total genomic DNA from ML larvae was isolated using a tissue DNA Purification Kit (EURx, Poland) according to the kit instructions. Following Appleyard et al. [10] we used primers oTSR1 and oTSR4 to differentiate between species based on partially conserved and repetitive genomic DNA sequences that are variable in length within the different *Trichinella* genotypes.

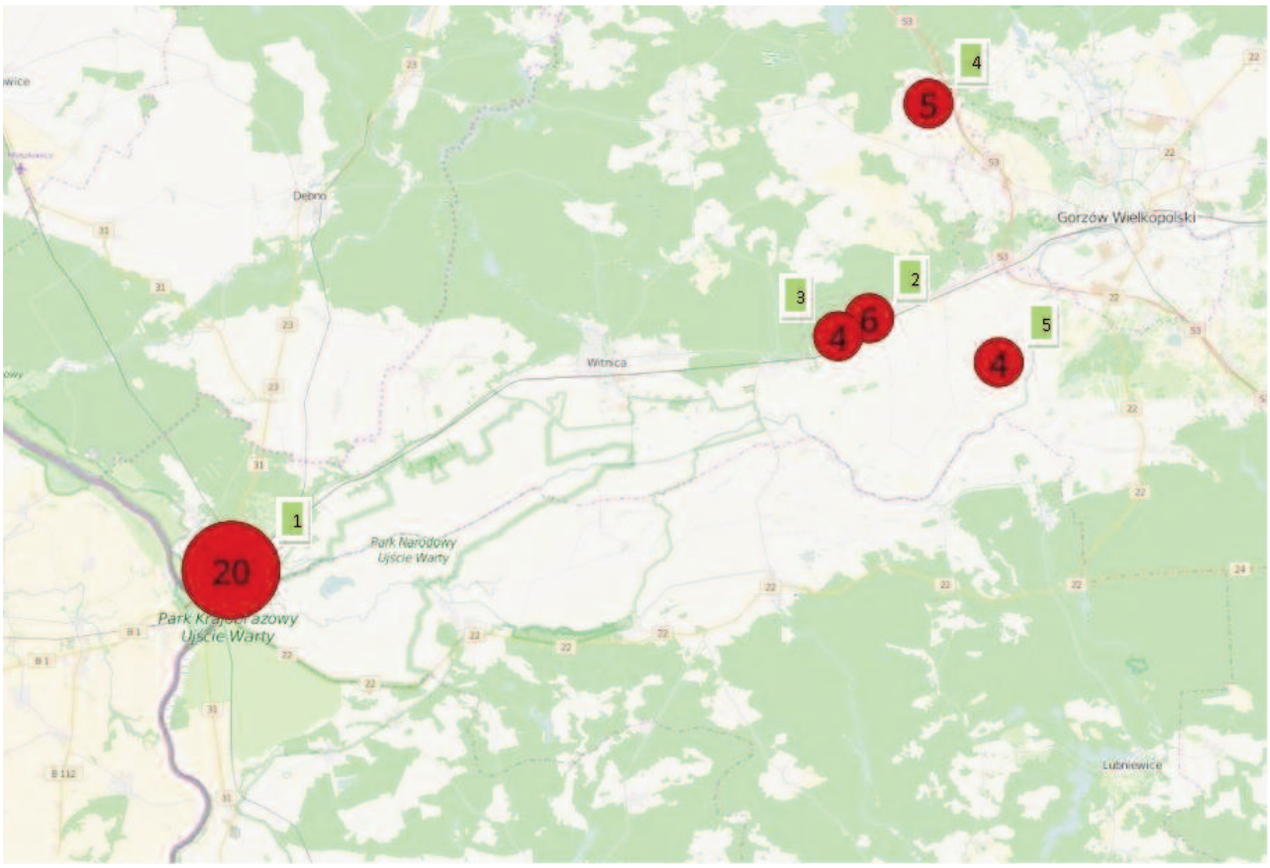

Fig. 1. Map of location of five sampling sites in western Poland 1– Warta Mouth National Park; 2 – Bogdaniec; 3 – Motylewo; 4 – Marwice; 5 – Jasiniec

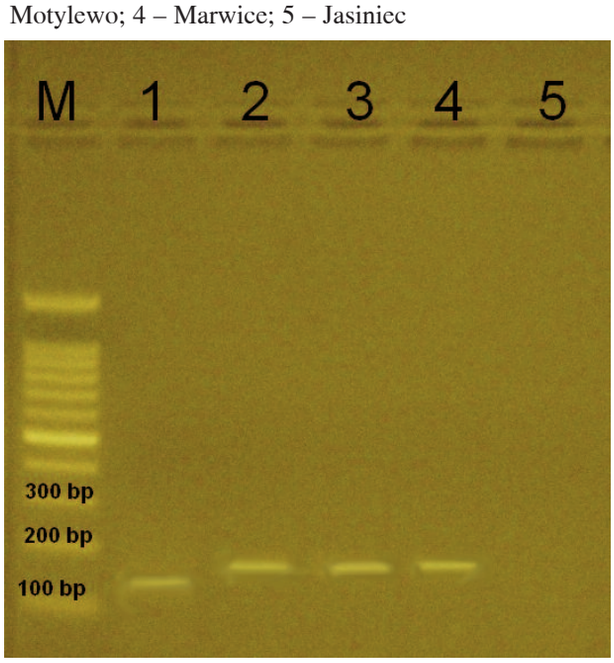

Fig. 2. The amplification products of *Trichinella* conserved 28S ribosomal RNA from larvae
Explanation: M – molecular weight standard 100–1000 (Novazym); 1 and 2 – positive controls of *T. britovi* and *T. spiralis* with bands 125 bp and 175 bp, respectively; 3 and 4 – positive samples of *T. spiralis* originating from two a raccoon dogs J2 and J5, respectively; 5 – negative control.

Amplification was done in a 25µl final volume of the following reagents: 2.5 mM $MgCl_2$, 0.6–1 µM of each primer, 0.2 mM of each deoxynucleotide triphosphate, and 0.5 U of Ampli*Taq* Gold DNA polymerase. Two reference strains of *T. spiralis* (ISS 3) and *T. britovi* (ISS 384) were used as a positive control, whereas the negative control was a reaction mixture without the DNA template. Polymerase Chain Reactions were performed in a GeneAmp 2400 thermocycler. The partial conserved 28S ribosomal RNA fragment was used as molecular marker. Reference isolates of *T. spiralis* and *T. britovi* had unique patterns, with bands migrating at approximately 175 bp, and 125 bp, respectively [10]. The amplicons were analysed on 2% agarose gels. The gel was visualised and analysed under UV in 320 nm light using the Documentation Gel (PhotoPrep I- Photodyne Electrophoresis).

Results and Discussion

The larvae were identified as *Trichinella* based on their morphology an identified at species level by genetic methods. Only two raccoon dogs J2 and J5 sampled in the Forestry Bogdaniec (0.8%) were

infected with *Trichinella*, which genetic analyses showed to be *T. spiralis* (Fig. 2). The raccoon dogs J2 and J5 harboured 0.02, and 0.06 LPG, respectively. In the present study, the infection rate in raccoon dogs was low. However, higher prevalence of *Trichinella* infection in raccoon dogs was found in other regions of Poland (24.4%) [11].

Up to date, two *Trichinella* species; *T. spiralis* and *T. britovi* were detected among wild raccoon dogs in Poland [11]. The detection of a *T. spiralis* infecting wildlife population of raccoon dogs affirms the circulation possibility of the parasite among domestic and wild animals.

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