SPECIES IDENTIFICATION OF TRICHINELLA LARVAE USING POOLED SAMPLES IN MULTIPLEX PCR

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Introduction

Trichinella infections are common in Finnish wildlife. In the years 2014 and 2015 34 % and 22 % of wild animals were positive in the surveillance program for trichinellosis. The most common species is cold-resistant *Trichinella nativa (T2)*. *Trichinella britovi* (T3) and *T. pseudospiralis* (T4) also occur while *T. spiralis* (T1) is rare. The occurrence of these four species among *Trichinella* positive wild animals were 0.5 %, 93.6 % 8.4 %, and 3.5 % (n= 314) in 2014 and 0 %, 88.5 %, 9.2 %, and 2.3 % (n=218) in 2015 for T1, T2, T3 and T4, respectively.

Approximately 5 % of the samples contain 2-3 *Trichinella* species. European Union Reference Laboratory (EURL) for parasites recommends multiplex PCR method for species identification and suggests examination of five larvae individually from each animal. However, five PCR test runs for all positive animals makes the surveillance of trichinellosis laborious and expensive. We tested the species identification using a pooled sample of 4-5 *Trichinella* larvae in one PCR test run.

Materials and methods

Trichinella larvae were lysed with proteinase K treatment and DNA was captured on magnetic beads based method (Promega DNA IQ System). Following multiplex PCR with 5 oligonucleotide primerpairs allowed identification of *Trichinella* species by one amplification assay. Six mixed samples (Table 1) were tested using EURL instructions for species identification.

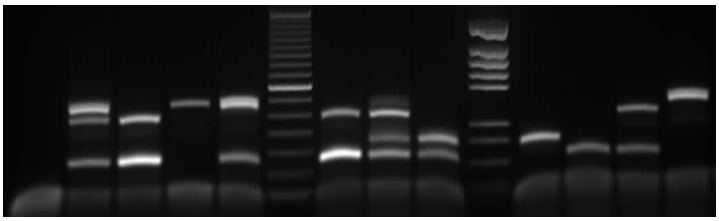
Table 1. The number of larvae of four *Trichinella* species in six samples examined for species identification by multiplex PCR.

Sample	1	2	3	4	5	6
T. spiralis					1	2
T. nativa		4	1	4	1	3
T. britovi	1	1		1	1	
T. pseudospiralis	4		3		1	

Results

The PCR products of each *Trichinella* species were detected in all mixed samples (Figure 1). However, for *Trichinella britovi* the amplification bands are of 127 bp and 253 bp, while for *T. nativa* there is only one product of 127 bp. When *T. britovi* was present in a pooled sample, it was necessary to repeat the examination by testing individual larvae to confirm the absence of *T. nativa.* Nevertheless, the number of test runs was remarkably lower when pooled samples instead of single larvae were used for species identification (Table 2).

Figure1. Multiplex PCR analysis of *Trichinella* mixed samples. Single larvae of *T. spiralis*, *T. nativa*, *T britovi* and *T. pseudospiralis* were used as controls.



PCR-mix	Mix 1	Mix 2	Control	Mix 3	50 bp ladder	Mix 4	Mix 5	Mix 6	Bench Top Marker	Controls			
	T3,T4	T2,T3	T4	T2,T4		T2,T3	T1,T2, T3,T4	T1,T2		T1	T2	T3	T4

Table 2. The number of PCR runs when either five single larvae per infected animal or a pooled sample of five larvae were used for the species identification for the surveillance of trichinellosis in Finnish wildlife in 2014 and 2015.

Year	2014	2015
Single larvae	1570	1090
Pooled sample	444	318

Conclusion

The use of pooled samples of five larvae for the species identification seems to be a practical and useful method for the surveillance of trichinellosis in wildlife. However, in the mixed infections of *Trichinella britovi* and *T. nativa* the latter remains unidentified. When *T. britovi* is detected in a pooled sample, the species identification should be repeated by testing individual larvae.