

Muscle distribution of sylvatic and domestic *Trichinella* larvae in production animals and wildlife

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Abstract

Only a few studies have compared the muscle distribution of the different *Trichinella* genotypes. In this study, data were obtained from a series of experimental infections in pigs, wild boars, foxes and horses, with the aim of evaluating the predilection sites of nine well-defined genotypes of *Trichinella*. Necropsy was performed at 5, 10, 20 and 40 weeks post inoculation. From all host species, corresponding muscles/muscle groups were examined by artificial digestion. In foxes where all *Trichinella* species established in high numbers, the encapsulating species were found primarily in the tongue, extremities and diaphragm, whereas the non-encapsulating species were found primarily in the diaphragm. In pigs and wild boars, only *Trichinella spiralis*, *Trichinella pseudospiralis* and *Trichinella nelsoni* showed extended persistency of muscle larvae (ML), but for all genotypes the tongue and the diaphragm were found to be predilection sites. This tendency was most obvious in light infections. In the horses, *T. spiralis*, *Trichinella britovi*, and *T. pseudospiralis* all established at high levels with predilection sites in the tongue, the masseter and the diaphragm. For all host species, high ML burdens appeared to be more evenly distributed with less obvious predilection than in light infections; predilection site muscles harbored a relatively higher percent of the larval burden in light infections than in heavy infections. This probably reflects increasing occupation of available muscle fibers as larger numbers of worms accumulate. Predilection sites appear to be influenced primarily by host species and secondarily by the age and level of infection.

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1. Introduction

The muscle distribution of the different *Trichinella* genotypes in a given host is not only important for

meat inspection of production animals, where sylvatic *Trichinella* species are occasionally found, but also for epidemiological surveillance in indicator animals, e.g. foxes and wild boars. In this study, muscle tissue was obtained from a series of experimental infections in pigs, wild boars, foxes and horses, with the aim of evaluating the predilection sites of 9 well-defined genotypes of *Trichinella*.

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2. Materials and methods

2.1. Parasitological material

Nine genotypes of *Trichinella*, all registered at the International *Trichinella* Reference Centre (TRC) in Rome, were used for inoculations: *Trichinella spiralis* (T1) (ISS004, Maryland, USA, domestic pig), *Trichinella nativa* (T2) (ISS042, Alaska, *Ursus maritimus*), *Trichinella britovi* (T3) (ISS100, Italy, *Canis lupus*), *Trichinella pseudospiralis* (T4, USSR) (ISS013, Caucasus, USSR, *Procyon lotor*), *T. pseudospiralis* (T4, USA) (ISS470, Alabama, USA, *Coragyps atratus*), *T. pseudospiralis* (T4, AUST) (ISS141, Australia, *Dasyurops maculatus*), *Trichinella murrelli* (T5) (ISS035, Pennsylvania, USA, *Ursus americanus*), *Trichinella* sp. (T6) (ISS034, Montana, USA, *Ursus arctos*), *Trichinella nelsoni* (T7) (ISS037, Tanzania, Africa, *Phacochoerus aethiopicus*). The parasites were propagated by serial passage in outbred Ssc:CF1 mice. Muscle larvae (ML) used for the inoculation of pigs were recovered by digestion and sedimentation as described below.

2.2. Experimental animals

Muscle samples were obtained from a total of 102 pigs, 36 wild boars, 30 horses and 108 foxes and necropsy was performed at 5, 10, 20, and 40 weeks post inoculation. All animals were treated in accordance with the animal ethics laws of Denmark.

2.3. Larval recovery and tissue digestions

From all host species, the number of ML was determined in corresponding muscles/muscle groups by artificial digestion according to Kapel and Gamble (2000). The muscles sampled were: the diaphragm (lumbar and costal parts), the tip and base of tongue (m. lingua anterior/posterior), the lower jaw (m. masseter), the abdomen (m. rectus abdominus), the tenderloin (m. psoas minor), the neck (m. splenius), the shoulder (m. trapezius), the throat (m. sternohyoideus), the upper forelimb (m. biceps-/m. triceps brachii), the lower forelimb (flexor digitorum), the upper hindlimb (m. quadriceps), the lower hindlimb (m. gastrocnemius), the upper jaw (m. temporalis), the intercostals (m. intercostales), the rump (m. gluteus

maximus/medius), the filet (m. longissimus dorsi). To compare predilection sites in animals with high and low infection levels, a score (*I*%) indicating the relative larval burden was calculated for each muscle group, using the highest number of larvae per gram (lpg) in each animal as the 100% reference point (Table 1). This value was used for statistical comparison of particular host–parasite combinations and finally to rank muscle larvae intensity in the examined muscles.

A rough estimate of digestibility of the different muscle tissues was performed on 20 g samples of surplus tissues (except for fox tongue where there was no extra tissue). For this, each tissue sample was minced into 3 mm pieces in a grinder and digested individually at 45 °C in 500 ml digestion fluid (500 ml H₂O, 5 ml HCl, 5 g pepsin (1:10.000 NF)) in a 1000 ml beaker on a heated magnetic stirrer with digital reading of temperature in the fluid. At 30 min intervals, the fluid was poured through a 200 µm sieve into another pre-heated beaker (45 °C) and the amount of any retained tissue was determined. If more than 0.2 g tissue was retained, the tissue was returned into the digestion fluid for another 30 min. Complete digestion was defined as having less than 0.2 g of tissue retained on the sieve.

2.4. Statistical analysis

The rank of relative larval burden (*I*%) of muscle tissues in different host parasite combinations was compared by a Friedman two-factor analysis of variance (Campbell, 1974). Comparisons were done according to host species, age of infection, infection level, and encapsulating versus non-encapsulating species.

3. Results

When comparing the ranking of infection levels in different muscles for particular host parasite combinations, no significant differences were found for the respective *Trichinella* species in pigs, wild boars, or horses (Table 1). In contrast, there was a significant difference in the tissue distribution of encapsulating and non-encapsulating species in foxes. No statistical

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