

Influence of infection intensity on predilection sites in swine trichinellosis

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Abstract

The muscular distribution of *Trichinella spiralis* or *T. britovi* was studied by digestion in 59 experimentally infected pigs and seven wild boars. Crus muscle was the predilection site in 89.3% of 28 heavily infected swine with 146–3634 larvae per gram (lpg), but in 51.6% of middle to light infections (0.005–59 lpg) the basis of the tongue showed higher larval densities than the crus muscle. The basis of the tongue was also the predilection site in 71.4% of wild boars. Highest counts in other muscles were found only in lightly infected pigs. The influence of intensity of infection, host species, and *Trichinella* species on muscle distribution is discussed.

Introduction

Most naturally infected animals with *Trichinella* harbour light infections (Schad *et al.*, 1985), and the diagnostic sensitivity of trichinelloscopy and pooled digestion method (Zimmerman, 1974) would be very low if other than predilection muscles are examined.

Materials and methods

Predilection sites of *Trichinella* spp. were determined in 59 experimentally infected pigs and seven naturally infected wild boars (table 1). The species of parasite producing the infections were determined by random amplified polymorphic DNA (RAPD) and/or allozyme analysis at the *Trichinella* Reference Center (Pozio *et al.*, 1989). Samples of crus muscle (CM), basis of tongue (BT), masseters (MM) and intercostal muscles (IM) were examined in all the infected hosts. In addition, the flexor-extensor forearm muscles were also examined in the wild boars and in 28 highly infected pigs. In the remaining 31 pigs the following muscles were examined

in addition: the abdominal muscles (straight, internal and external oblique), brachial biceps, semitendinous, back longest, psoas major and seven anatomical portions of the diaphragm (the right and left crus muscle, lumbocostal arch, dorsal costal part, ventral costal part, sternal part and tendinous centre). Initially, 5 g muscle samples freed from fat and connective tissues were examined by HCl-pepsin digestion (Serrano *et al.*, 1992). If samples were negative or only a few larvae obtained (<300 larvae), additional muscle tissues or even entire muscles were digested. The mean of larvae per gram (lpg) in CM, BT, MM and IM was considered as the infection level (IL) of each animal. A relative percentage of infection (RPI) for each muscle was also calculated, using the IL as a 100% reference value. The Friedman two-way analysis of variance (ANOVA), the Pearson linear correlation test, and the Kruskal-Wallis ANOVA were used for analysing the data.

Results and Discussion

Differences of RPI among CM, BT, MM and IM in all hosts were significant by the Friedman test ($P < 0.001$), except between CM and BT ($P = 0.086$). The predilection sites in wild boars were BT (71.43%) and CM (28.57%). In highly infected swine the predilection site was CM,

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Table 1. Distribution of *Trichinella* larvae in different muscles/muscle groups of heavily and lightly infected pigs and naturally infected wild boars.

	Low infected pigs (n=31) ^a				High infected pigs (n=28) ^b				Wild boars (n=7) ^a			
	x	Median	Range	SD	x	Median	Range	SD	x	Median	Range	SD
Infection level (IL)	15	10	0.005–59	17	947	826	146–3634	678	109	21	1.3–379	158
Doses (larvae per animal)	380	200	25–3750	670	4902	3750	1250–36000	6245				
RPI (%)												
Tongue (basis)	143	128	0–400	82	108	125	21–154	40	159	125	51–315	89
Diaphragm												
Crus muscle (<i>crus diafragmatis</i>)	126	116	0–278	56	163	159	131–222	20	110	87	53–231	61
Right crus muscle (<i>crus dextrum diafragmatis</i>)	131	125	0–366	73								
Left crus muscle (<i>crus sinistrum diafragmatis</i>)	117	121	0–284	62								
Sternal part (<i>pars sternalis</i>)	104	75	0–364	97								
Ventral costal part (<i>pars costalis</i>)	88	89	0–180	55								
Dorsal costal part (<i>pars costalis</i>)	86	98	0–165	46								
Lumbocostal part (<i>arcus lumbocostalis</i>)	74	62	0–199	58								
Tendinous center (<i>centrum tendineum</i>)	44	36	0–156	38								
<i>Psoas major</i>	88	73	0–491	89								
<i>Masseter</i>	77	71	0–400	69	78	73	39–143	22	86	56	23–180	57
Abdomen												
Internal oblique (<i>obliquus internus abdominis</i>)	83	64	0–527	91								
Straight abdominal (<i>rectus abdominis</i>)	83	60	0–618	110								
External oblique (<i>obliquus externus abdominis</i>)	50	52	0–124	31								
Intercostal muscles (<i>intercostalis</i>)	54	44	0–171	44	51	38	23–137	31	45	49	9–76	23
Forearm (flexor/extensor muscles)					45	45	4–87	17	66	56	25–110	36
Brachial biceps (<i>biceps brachii</i>)	44	42	0–121	29								
Back longest (<i>longissimus dorsi</i>)	35	29	0–124	25								
Semitendinous (<i>semitendinosus</i>)	35	36	0–128	25								

^aOne host infected with *T. britovi*; ^bthree pigs infected with *T. britovi*; x, mean; SD, standard deviation; IL, average of larvae per gram in crus muscle, basis of tongue, masseter and intercostal muscles of each host; RPI, relative percentage of infection using IL in each host as reference equal to 100%.

Table 2. Comparison of the relative percentage of infection (RPI) in experimental infections with *Trichinella spiralis* and *T. britovi* in pigs.

Muscle	<i>T. spiralis</i> 23–459 lpg n=10 pigs	<i>T. britovi</i> 23–459 lpg n=4 pigs
Intercostal	29*	68*
Masseter	95	92
Diaphragm (crus muscle)	114	173
Tongue (basis)	162*	67*

* Differences between RPI in tongue and intercostal muscles of the two parasite species are significant ($P < 0.05$, Kruskal-Wallis ANOVA).

lpg: larvae per gram.

except in three animals the highest counts were recorded in the BT. In pigs with low intensity of infection this predilection pattern was not as expressed and CM harboured the most larvae in only ten pigs, BT in 16, IM in three and MM in two. The lpg of CM, BT, MM and IM muscles were significantly correlated with the IL (Pearson linear correlation, $P < 0.05$; $0.99 < r > 0.44$). Considering the relative values of larval intensity, only CM correlated with IL at a significance level ($r = 0.26$) whereas no correlation could be demonstrated between BT and IL ($r = -0.15$). The differences of RPI between CM and BT in highly infected pigs were clearly significant ($P = 0.0003$), but were minimal in lightly infected pigs ($P = 0.715$). According to RPI values of other muscles in this group, the predilection site order was the BT (38.7%), CM (22.6%), sternal part of diaphragm (19.3%), IM (6.4%), MM, psoas major, lumbocostal arch and costovertebral part of diaphragm (3.2% each). In two cases, only a few larvae were found in the BT or MM respectively, despite the fact that at least 600 g of muscle were examined in each host.

The predilection sites of *Trichinella* larvae clearly depend on the host species. These are tongue or masseters in horses (Soulé *et al.*, 1989; Gamble *et al.*, 1996), forearm muscles in carnivores (Kapel *et al.*, 1994, 1995), and masseters in ruminants (Reina *et al.*, 1996). Wild boars and domestic pigs, still the source of many human outbreaks, have been studied earlier (Zimmermann, 1970; Kotula *et al.*, 1984; Piergili Fioretti *et al.*, 1994; Kapel *et al.*, 1998) but only limited information has been presented on how the intensity of infection and host genetics influence muscle distribution. Olsen *et al.* (1964) and Christensson (1994) found that the diaphragm of pigs was heavily infected whereas Hill (1968) and Lizcano-Herrera (1979) indicated that tongue had more larvae in pigs harbouring light infections. Among 46 swine, Köhler (1984) found a similar degree of infection in these muscles (the lpg in the tongue was 99.45% with respect to the diaphragm) although diaphragm was the predilection site in 29 hosts. On the contrary, in a similar study on 31 swine, Kotula *et al.* (1988) found that infection of the tongue was 116% with respect to the diaphragm.

The species of parasite appears to influence the muscle distribution (table 2). In low to moderate infections, the BT was found to be more significantly infected in *T. spiralis* infections ($P < 0.05$, Kruskal-Wallis ANOVA), whereas

the CM appears to be the more apparent predilection site in *T. britovi* infections. This pattern could not be demonstrated by Kapel *et al.* (1998), but different levels of infections of pigs were compared by these authors. Isolates of both species were also compared by Franchimont *et al.* (1998) with unclear results since only significant larval counts were found in the tongue, and therefore further studies are needed to evaluate the importance of species of *Trichinella*.

Christensson (1994) reported that the level of infection in swine can influence the muscle distribution of *Trichinella*. In horses, Gamble *et al.* (1996) found that the tongue harbours more larvae than the masseters in light infections (< 100 lpg). This variability can be explained in part by the sampling method. Worm burdens can differ markedly in the same muscle, for instance, between the basis and the tip of the tongue (Kapel *et al.*, 1994) or amongst parts of the diaphragm (Kotula *et al.*, 1988 and the present study). Nevertheless, intensity-dependent distribution of larvae can be explained if newborn larvae are passively carried by the blood flow and only those establishing in myofibres surrounded by capillary networks of venous arborizations can survive (Wright *et al.*, 1989). In heavy infections, the larval distribution is likely to reflect the distribution of available sites. However, in lower infections, the saturation of muscles must play a secondary role, relying mostly on the amount of blood flow in each muscle. This can explain the higher density of larvae in BT in light infections.

From a practical point of view, the distribution in highly infected swine has negligible importance since most muscles have detectable numbers of larvae. Unfortunately, experimental swine infections often yield high larval burdens, unlike low natural intensities of infection. Thus, predilection sites might need further attention. It is worth noting that in lightly infected swine, BT appears to be as reliable as CM, but MM and IM, often considered predilection sites (Van Knapen *et al.*, 1996), are poorly infected (Lizcano-Herrera, 1979; Köhler, 1984; Kotula *et al.*, 1984; Prost and Nowakowski, 1990).

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