Acta vet. scand. 1994, *35*, 125-132.

A Study on the Predilection Sites of *Trichinella spiralis* Muscle Larvae in Experimentally Infected Foxes *(Alopex lagopus, Vulpes vulpes)*

By *Chr. M. Kapel*[1], *Sv. Aa. Henriksen*[2], *H. H. Dietz*[3], *P. Henriksen*[3] and *P. Nansen*[1]

[1]Danish Centre for Experimental Parasitology, Royal Veterinary and Agricultural University, Frederiksberg and National Veterinary Laboratory[2] Copenhagen and[3] Aarhus, Denmark.

Kapel, Chr. M., Sv. Aa. Henriksen, H. H. Dietz, P. Henriksen, P. Nansen: A study on the predilection sites of *Trichinella spiralis* muscle larvae in experimentally infected foxes (Alopex lagopus, Vulpes vulpes). Acta vet. scand. 1994, 35, 125-132. – Studies were carried out on the predilection sites of *Trichinella spiralis* muscle larvae in experimentally infected arctic foxes *(Alopex lagopus)* and silver foxes *(Vulpes vulpes)* reared in cages. The highest number of larvae per gramme tissue was found in the muscles of the legs, eyes, diaphragm, and tongue. The 2 fox species showed no significant differences with regard to predilection sites.

nematodes; distribution; diagnosis, carnivarous host.

Introduction

Predilection sites of muscle larvae of *Trichinella spiralis* depend upon the host species *(Borchert* 1962). However, only few reports exist on the predilection sites of *T. spiralis* larvae in carnivores *(Hermansson* 1943, *Fassbender & Meyer* 1974, *Larsen & Kjos-Hansen* 1983).

For the *post-mortem* diagnosis of *T. spiralis* infections, it is of importance, in particular in field situations, to know the predilection sites of the parasite, as this will make the diagnosis more sensitive.

The present paper describes results obtained by experimental infections of 12 foxes.

Materials and methods

Eight male, arctic foxes *(Alopex lagopus),* 8 months old, and 4 male, silver foxes *(Vulpes vulpes),* 12 months old, raised in cages and fed dry pellets and water *ad lib.,* were inoculated with larvae of *T. spiralis* by stomach tubes. The larvae originated from guinea pigs inoculated 3 months earlier. The strain of *T. spiralis* used in this study has been maintained in guinea pigs for more than 15 years, and has been identified as T_1 *(T. spiralis, sensu stricto)* at the Trichinella Reference Center in Rome, Italy. Four arctic foxes and 2 silver foxes were inoculated with 500 larvae each, while the remaining 6 animals were inoculated with 5,000 larvae each.

Six weeks after the inoculation, the foxes were sacrificed, and 5-15 g tissue samples from 18 selected muscles/muscle groups were examined (Table 1). In larger muscles/muscle groups, tissue samples were taken from the part of the muscle closest to the tendon. In smaller muscles/muscle groups, all muscular tissue was included in the samples. The individual samples freed from tendons and fasciae were minced by means of a pair of scissors, and subsequently examined using a combined digestion and Baermann technique *(Henriksen* 1973).

To measure the distribution of larvae in animals with different levels of infection, a relative value of larval burden was calculated for each animal, using the muscle with the highest number of larvae per g tissue (l/g) in that particular animal as a reference, equal to 100%. For each muscle group, the relative values were summarized for all animals and a mean was calculated (Table 1). Analysis of variance was used for processing the data in SAS (Statistical Analysis System) *(SAS Institute Inc 1985)*.

During the experimental period all the animals were observed for clinical symptoms.

Results

No clinical symptoms were observed following the inoculation with *T. spiralis* larvae. In 1 silver fox inoculated with 500 larvae infection failed to establish. The digestion technique yielded positive results for almost all the muscle groups examined (97%) of the remaining 11 animals. The larval densities in the selected muscle groups are summarized in Table 1. The level of infection varied considerably in between the foxes, as the average number of larvae per gramme tissue showed values from 2 to 291. The number of muscle larvae did not exceed 660 per gramme in any of the samples examined.

The distribution of larvae in the examined muscles/muscle groups is illustrated in Fig. 1, where relative values of larval burdens are compared. The highest relative larval densities were found in the following muscles/muscle groups: The muscles of the eye *(m. rectus dorsalis/medialis/lateralis/ventralis + m. obliquus dorsalis/ ventralis),* the lower part of the frontleg *(m. flexor carpi ulnaris/m. extensor carpi radialis),* the hindleg *(m. gastrocnemius),* the tongue *(m. lingualis proprius),* and the diaphragm *(m. pars lumbalis/pars costalis).*

No significant differences between the 2 fox species *V. vulpes* and *A. lagopus* could be demonstrated with regard to the predilection sites (two-factor analysis of variance, p>0.05). Even though the high inoculation dose (5,000 larvae) gave a higher average number of larvae/g, this tendency is not significant (two-factor analysis of variance, p>0.05).

Discussion and conclusion

When comparing the muscle larval distribution of *T. spiralis* in different host species, the number of larvae per g muscle tissue seems to be related to the potential for motoric activity patterns and blood flow of the particular muscle in that particular host. These predilection differences between host species seem apparent from the available literature. The present investigation lends support to this distribution characteristics.

Most experimental work on predilection sites of *T. spiralis* has been performed on rodent host species. In a study on laboratory rats, the diaphragm, the tongue, the jaw muscles, and the lower part of the legs were found to be the most heavily infected muscles *(Hoefelschweiger* 1989). In laboratory mice, *Stewart & Charniga* (1980) found the *m. masseter*, the *m. biceps,* and the diaphragm most heavily infected whereas the tongue and the abdominal muscles had lower larval densities. A comparable pattern has been observed among other rodent species, e.g. also in experimentally infected rabbits, the muscle groups of the ear, jaw, eye, tongue, and diaphragm were most heavily infected *(Henriksen* 1980). In experimentally infected sheep, the *m. masseter* was the heaviest infected muscle followed by the muscles of the tongue and the diaphragm, while the lowest infection levels were found in

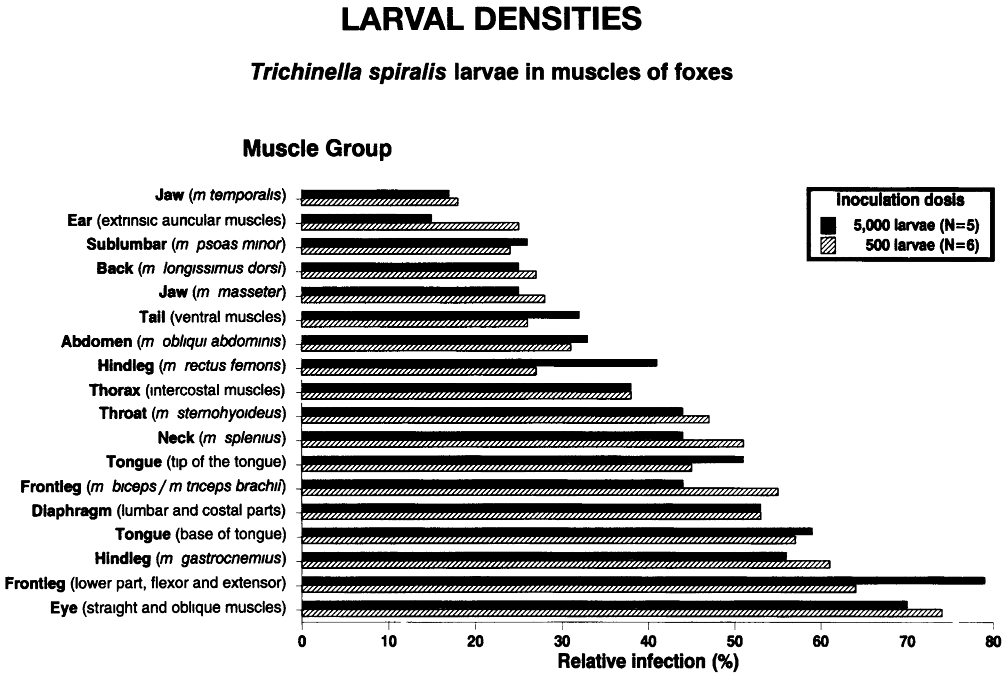

Figure 1. Relative values of the number of *Trichinella spiralis* muscle larvae in selected muscles/muscle groups from 8 arctic foxes and 3 silver foxes.

the *m. psoas minor, m. rectus abdominis,* and the intercostal musculature *(Smith & Snowdon* 1989). Similarly, in another study on experimentally infected sheep *(Alkarmi et al.* 1990), high levels of infection were demonstrated in the diaphragm and tongue, while only low infection levels were found in the muscles of the limbs, but unfortunately muscles of the jaw were not examined. In experimentally infected cattle, predilection sites were found to be the *m. masseter,* the tongue, and the diaphragm *(Smith et al.* 1990). Thus the results from experiments on herbivorous host species may reflect some specialized use of the jaw muscles in the processing of plant material.

In carnivorous host species the predilection sites seem to be somewhat different. In a study of polar bears, *T. spiralis* larvae were less frequently demonstrated in the *m. masseter* (18%) compared to the diaphragm (33%), and none of the animals were infected in the *m. masseter* only *(Larsen & Kjos-Hanssen* 1983). Likewise, *m. masseter* was found to be less infected than the diaphragm, tongue, and intercostal muscles in experimentally infected dogs *(Martinez-Gómez et al.* 1980). *Fassbender & Meyer* (1974) examined the distribution of *T. spiralis* muscle larvae in 3 species of naturally infected North African wild carnivores: jackal *(Canis aureus),* mongoose *(Herpestes ichneumon),* and genet *(Genetta genetta).* The analyses revealed substantial infection of the muscles of the distal limb. This was suggested to be related to a high running pensum searching a big homerange for prey. A similar larval distribution was found by *Hermansson* (1943) in 2 red foxes caught wild

Table 1 Number of *Trichinella spiralis* muscle larvae in selected muscles/muscle groups from 8 arctic foxes and 3 silver foxes.

	Larval densities Low Dose (500 larvae)													
	Fox A1		Fox A2		Fox A3		Fox A4		Fox V1		Mean			
	l/g	I%	l/g	I%	l/g	I%	l/g	I%	l/g	I%	l/g	S.D.	I%	S.D
*** Eye (straight and oblique muscles)	45	100	14	53	13	23	69	92	20	100	32	24	74	34
*** Frontleg (lower part, flexor and extensor)	35	77	26	100	56	100	75	100	7	35	40	26	64	40
** Hindleg *(m.gastrocnemius)*	32	70	19	73	27	48	64	85	6	30	30	22	61	22
** Tongue (base of the tongue)	23	50	19	71	23	41	49	65	12	60	25	14	57	12
** Diaphragm (lumbar and costal parts)	45	100	18	67	14	25	33	44	6	30	23	16	53	31
** Frontleg *(m biceps brachii/m.triceps brachii)*	32	72	18	69	19	34	50	67	7	35	25	16	55	19
** Tongue (tip of the tongue)	20	44	22	82	19	34	68	51	3	15	20	12	45	25
** Neck *(m splenius)*	31	67	23	87	21	68	61	41	4	20	22	11	51	26
** Throat *(m.sternohyocideus)*	28	62	18	67	20	36	30	40	6	30	20	10	47	16
** Thorax (intercostal muscles)	26	57	15	59	15	27	22	29	4	20	16	8	38	18
** Hindleg *(m.rectus femoris)*	14	30	13	49	8	14	24	32	2	10	12	8	27	16
* Abdomen *(m.obliqui abdominis)*	18	40	7	28	16	29	28	37	4	20	15	10	31	8
* Tail (ventral muscles)	19	42	8	32	15	27	19	25	1	5	12	8	26	14
* Jaw *(m.masseter)*	14	31	10	39	9	16	24	32	4	20	12	7	28	9
* Back *(m longissimus dorsi)*	14	30	14	51	11	20	13	17	3	15	11	5	27	15
* Sublumbar *(m.psoas minor)*	12	26	10	37	13	23	18	24	2	10	11	6	24	10
* Ear (extrinsic auricular muscles)	13	29	6	23	6	11	35	47	3	15	8	6	18	8
* Jaw *(m.temporalis)*	12	27	6	22	4	7	16	21	3	15	8	6	18	8
Mean	24	53	15	56	17	31	35	47	5	27	*19.4*	*15.0*	*42.8*	*25.4*

Fox A1-A8: *Alopex lagopus*
Fox V1, V3, V4: *Vulpes vulpes*

*** High degree of infection (>60%)
**: Medium degree of infection (30-60%)
* Low degree od infection (<30%)

l/g: Larvae per gramme
I %: Relative infection
S.D.: Standard deviation

Table 1 (continued). Number of *Trichinella spiralis* muscle larvae in selected muscles/muscle groups from 8 arctic foxes and 3 silver foxes.

	Larval densities High Dose (5,000 larvae)															
	Fox A5		Fox A6		Fox A7		Fox A8		Fox V3		Fox V4		Mean			
	l/g	I%	l/g	I%	l/g	I%	l/g	I%	l/g	I%	l/g	I%	l/g	S D	I%	S D.
*** Eye (straight and oblique muscles)	37	48	39	68	106	85	660	100	17	100	1	20	143	256	70	32
*** Frontleg (lower part, flexor and extensor)	78	100	57	100	125	100	624	95	7	41	2	40	149	237	79	30
** Hindleg *(m.gastrocnemius)*	31	40	31	55	95	76	383	58	5	29	4	80	92	147	56	20
** Tongue (base of the tongue)	34	44	32	57	61	49	523	79	8	47	4	80	110	203	59	16
** Diaphragm (lumbar and costal parts)	39	50	53	94	64	51	224	34	8	47	2	40	65	82	53	21
** Frontleg *(m biceps brachii/m.triceps brachii)*	40	52	15	26	45	36	374	57	6	35	3	60	81	145	44	14
** Tongue (tip of the tongue)	25	33	23	41	49	39	431	65	5	29	5	100	90	168	51	27
** Neck *(m.splenius)*	42	54	32	56	33	26	323	49	3	18	3	60	73	124	44	17
** Throat *(m sternohyocideus)*	33	43	20	36	40	32	264	40	6	35	4	80	61	100	44	18
** Thorax (intercostal muscles)	25	32	25	45	43	34	166	25	5	29	3	60	45	61	38	13
** Hindleg *(m rectus femoris)*	45	58	14	25	44	35	336	51	3	18	3	60	74	130	41	18
* Abdomen *(m.obliqui abdominis)*	26	33	28	49	44	35	133	20	3	18	2	40	39	49	33	12
* Tail (ventral muscles)	8	10	26	49	28	22	213	32	7	41	2	40	47	82	32	14
* Jaw *(m masseter)*	16	21	12	21	61	49	146	22	3	18	1	20	40	56	25	12
* Back *(m.longissimus dorsi)*	34	43	16	29	32	26	57	9	4	24	1	20	24	21	25	11
* Sublumbar *(m.psoas minor)*	29	37	25	44	31	25	150	23	1	5	1	20	40	56	26	14
* Ear (extrinsic auricular muscles)	15	19	2	3	24	19	85	8	3	18	1	20	22	32	15	7
* Jaw *(m.temporalis)*	17	21	7	12	28	22	134	20	1	5	1	20	31	51	17	7
Mean	32	41	25	45	53	42	291	43	5	26	2	41	*68,0*	*125,1*	*41,8*	*24,0*

Fox A1-A8: *Alopex lagopus*
Fox V1, V3, V4: *Vulpes vulpes*

*** High degree of infection (>60%)
**· Medium degree of infection (30-60%)
*. Low degree od infection (<30%)

l/g: Larvae per gramme
I %: Relative infection
S.D.: Standard deviation

and 1 silver fox reared in a cage. It was found that the muscles of the front- and hindleg were heavily infected, while the diaphragm was only moderately infected and no larvae were observed in the muscles of the jaw. The larval distribution was found to be similar in all 3 foxes. Thus, contrary to the herbivorous host species the predilection sites of the carnivorous host species seem to be the extremities, which in turn could be interpreted as a muscular adaptation to intensive locomotive behaviour in search and catch of prey.

In the omnivorous species the predilection patterns seem more diverse compared to the herbivorous and carnivorous species, respectively. The distribution of larvae in primates was investigated by *Nelson & Mukundı* (1962) who found that *m. biceps, m. masseter, m. tibialis anterior,* and *m. flexor carpi ulnaris* had higher levels of infection than the diaphragm. Similarly, a study on *Macaca ırus* monkeys revealed the highest levels of infection in the *m. masseter* and the tongue and lower infection in the diaphragm *(Kociecka et al.* 1980). In a study by *Zimmermann* (1970), experımentally infected pigs revealed high levels of infections in the diaphragm and tongue, and low levels of infections in the muscles of the legs. Similar distribution was found in pigs where the tongue and diaphragm revealed a substantıally higher density of larvae than the muscles of the legs *(Kotula et al.* 1984).

Thus, in most host species, the high larval density of the diaphragm, the tongue, and the eye may reflect a basic intensive use of these particular muscles.

In the present study, the highest infection levels were found in the muscles of the eyes, legs, tongue, and diaphragm. This larval distribution in the present, cage-reared foxes is comparable with that of other studies on wild carnivorous, but different from experimentally infected herbivorous and omnivorous species. Considering the muscles of the legs, the high larval densities, as found in other carnivorous species, seem to depend on the motoric potential rather than on the actual motoric activity of the host, and therefore independent of the host having access to move freely or being reared in cages. A hypothesis supporting this was previously put forward by *Henriksen* (1980). Experiments performed on laboratory mice showed a negative correlation between muscular activity and larval invasion in the leg muscles *(Kozar & Kozar* 1960). Whether these findings reflect physiological stress, changed blood flow or other mechanisms remains to be clarified, and *Hughes & Harley* (1977) showed that first-stage migratory larvae are susceptible to low-voltage stimuli, and they concluded contrary: 'there is a possibility that the larval predilection for more active muscles can be explained by this increased negativity' (reversed action potential of the cell membrane).

The present observations support the assumptıon that the relative density of *T. spiralis* larvae, in individual muscles of different genus/species of hosts, will depend on the functional importance to which the muscles are predisposed in that particular genus/species of host, regardless of wheather the muscles are frequently used or not. *Bagherı et al.* (1986) showed that *T. spiralis* in mice only invade "fast twitch" muscle fibers (fast oxidative glycolytic fibers and fast glycolytic fibers), while *T. pseudospiralis* invades both "fast twitch" and "slow twitch" muscle fibers (slow oxidative fibers). As even closely related host species have very different muscle fiber composition in the same muscle groups, as in mice *(Bagheri et al.* 1986) and rat *(Ariano at al* 1972), this may result in a different distribution of *T. spiralis* muscle larvae in different host species.

In conclusion, the present study has demonstrated, that the predilection sites of *T. spiralis* muscle larvae in experimentally infected foxes seem to be represented by the following muscles/muscle groups: Muscles of the eyes, the lower part of the front and hind legs, the diaphragm, and the tongue. Thus, from a practical as well as from a scientific point of view, it seems relevant primarily to include tissue samples from the legs, when carrying out prevalence studies on *T. spiralis* infections in foxes under field conditions.

References

Alkarmi T, Behbehani K, Abdou S, Ooi HK: Infectivity, reproductive capacity and distribution of *Trichinella spiralis* and *T pseudospiralis* larvae in experimentally infected sheep. Jpn.J.vet.Res. 1990, *38,* 139-146.

Ariano MA, Armstrong RB, Edgerton VR: Hind limb muscle fiber populations of five animals. J.Histochem.Cytochem. 1972, *21,* 51-55.

Bagheri A, Ubelaker JE, Stewart GL, Wood B: Muscle fiber selectivity of *Trichinella spiralis* and *Trichinella pseudospiralis.* J.Parasitol. 1986, *72,* 277-282.

Borchert A: Lehrbuch der Parasitologie fur Tierarzte. (Textbook on parasitology for veterinarians). Hirzel Verlag, Leipzig. 1962, 337-338.

Fassbender CP, Meyer P: Uber die Verteilung von *Trichinella spiralis* in der Muskulature einiger nordafrikanischer Carnivoren. (Distribution of *Trichinella spiralis* in the musculature of some North African carnivores). Dtsch. Tierarztl. Wschr. 1974, *12,* 273-296.

Henriksen SvAa: Demonstration and isolation of *Trichinella spiralis* by a combined digestion and Baermann technique. Acta vet. scand. 1973, *14,* 356-358.

Henriksen SvAa: Observations on the predilection sites of *Trichinella spiralis* larvae in experimentally infected rabbits. Proceedings of the fifth International Conference on Trichinellosis. ed: Kim, C. W. Reedbooks, England. 1980, 183-186.

Hermansson KA: Några erfarenheter vid mikroskopiske undersokning av ravkott på trikiner. (Some experiences in microscopic investigation of the flesh of fox for trichinas). Skand.Vet.Tidskr. 1943, *33,* 281-301.

Hoefelschweiger, H: Untersuchungen uber Entwicklungsstadien und Verteilung von Trichinellenkapseln in der Muskulatur von experimentell infizierten Ratten im Zeitraum von 15 bis 78 Wochen mit einer Ueberpruefung der Nachweisbarkeit verkalkter Stadien mit der amtlich vorgeschriebenen Pepsin-Salzsaeure Digestionsmethode. (Analyses of development and distribution of capsules of *Trichinella spiralis* in muscles of experimentally infected rats over a period of 15 to 78 weeks with proving the detection rate of calcified stages by the officially prescribed pepsin-hydrochloric digestion method.). Freie Univ. Berlin. Fachbereich Veterinärmedizin. Berlin. 1989. 182 p.

Hughes WL, Harley JP: Trichinella spiralis: Taxes of first-stage migratory larvae. Exp.Parasitol. 1977, *42,* 363-373.

Kociecka W, van Knapen F, Ruitenberg EJ: Trichinella pseudospiralis and *T. spiralis* infections in monkeys. I. Parasitological aspects. Proceedings of the fifth International Conference on Trichinellosis. ed: Kim, C.W. Reedbooks, England. 1980, 199-203.

Kotula AW, Murrell KD, Acosta-Stein L, Lamb L: Distribution of *Trichinella spiralis* larvae in selected muscles and organs of experimentally infected swine. J.Anim.Science. 1984, *58,* 94-98.

Kozar Z, Kozar M· Influence of muscular work and other factors on the course of the invasion of *Trichinella spiralis.* Wiad.Parazyt. 1960, *6,* 363-366.

Larsen T, Kjos-Hansen B: Trichinella sp. in polar bears from Svalbard, in relation to hide length and age. Polar Research. 1983, 1 n.s., 89-96.

Martinez-Gómez F, Lanchas-Rivero J, Carratero RC, Moreno-Montañez T: Experimental trichinellosis in the dog: behaviour of the larvae. Proceedings of the fifth International Conference on Trichinellosis. ed: Kim, C.W. Reedbooks, England. 1980, 51-55.

Nelson GS, Mukundi J· The distribution of Trichinella spiralis in the muscles of primates. Wiad. Parazyt. 1962, *8,* 629-632.

SAS Institute Inc.: SAS Uder's guide, Statistics, Ver 6. edition. Cary, North Carolina USA. 1985.

Smith HJ, Snowdon KE: Experimental trichinosis in sheep. Can.J.Vet.Res. 1989, *53,* 112-114.

Smith HJ, Snowdon KE, Finley GG, Laflamme LF: Pathogenesis and serodiagnosis of experimental *Trichinella spiralis spiralis* and *Trichinella nativa* infections in cattle. Can.J.Vet.Res. 1990, *54,* 334-359.

Stewart GL, Charniga LM: Distribution of *Trichinella spiralis* in muscles of the mouse. J.Parasitol. 1980, *66,* 688-689.
Zimmermann WJ: Reproductive potential and muscle distribution of *Trichinella spiralis* in swine. J.Amer.Vet med.Ass. 1970, *156,* 770-774.

Sammendrag

Undersøgelse af prædilektionssteder for Trichinella spiralis larver i eksperimentelt inficerede ræve (Alopex lagopus, Vulpes vulpes)

Undersøgelser af prædilektionssteder for *Trichinella spiralis* muskellarver er foretaget i eksperimentelt inficerede polarræve *(Alopex lagopus)* og sølvræve *(Vulpes vulpes)* opvokset i fangenskab. Det største antal larver per gram væv blev fundet i ekstremitets- og øjenmuskulatur, samt i mellemgulv og tunge. De to rævearter viste ingen signifikant forskel med hensyn til prædilektionssteder.

(Received September 30, 1993; accepted December 12, 1993)

Reprints may be requested from: Chr. M. Kapel, Danish Centre for Experimental Parasitology, Royal Veterinary and Agricultural University, 13 Bulowsvej, DK-1870 Frederiksberg C, Denmark.