ISSN: 1220-3173; E-ISSN: 2457-7618

STUDY ON THE PREVALENCE AND LARVAL BURDEN OF THE NEMATODE *TRICHINELLA SPP*. IN RED FOXES FROM HUNTING GROUNDS IN TIMIS COUNTY

STUDIU PRIVIND PREVALENȚA ȘI DISTRIBUȚIA LARVARĂ A NEMATODULUI *TRICHINELLA SPP.* LA VULPILE ROȘII DIN FONDURI DE VÂNĂTOARE DIN JUDEȚUL TIMIȘ

Ana-Maria MARIN[1)], G. DĂRĂBUȘ[1)], V. HERMAN[1),*)], S. MORARIU[1)], T.R. OLARIU[2)], Rodica Georgiana DĂRĂBUȘ[2)], Beatrice Ana-Maria SÎRBU[1)], Narcisa MEDERLE[1)]

ABSTRACT

Trichinellosis is and remains one of the most important parasitic zoonoses, transmitted through the consumption of insufficiently cooked meat, meat from domestic and wild animals alike. In the context in which red foxes represent a sentinel of the intersection of the two domestic and sylvatic cycles, we followed in the study conducted in 2018-2020, the identification of the prevalence of trichinellosis in 34 red foxes from hunting grounds in Timis County. Following trichinelloscopy and artificial digestion, we established a prevalence of 28.12% and a maximum larval burden in the tongue muscles (59.10 larvae / gram), compared to the temporalis muscle (5.25 larvae / gram).

Keywords: *Trichinella spp.,* prevalence, larvae burden, red fox

REZUMAT

Trichineloza este și rămâne una dintre cele mai importante zoonoze parazitare, transmisă prin consumul cărnii insuficient preparată termic, carne provenită de la animalele domestice și sălbatice, în egală măsură. În contextul în care vulpile reprezintă o santinelă a intersecției celor două cicluri domestic și silvatic, am urmărit în studiul derulat în perioada 2018-2020, identificarea prevalenței trichinelozei la un număr de 34 de vulpi roșii provenite din fonduri de vânătoare din județul Timiș. În urma trichineloscopiei și digestiei artificiale, am stabilit o prevalență de 28.12 % și o distribuție larvară maximă în mușchii limbii (59.10 larve/gram), comparativ cu mușchiul temporal (5.25 larve/gram). Rezultatele conduc spre o atenționare asupra modalităților de transmitere și a relațiilor epidemiologice care se interferează în această importantă zoonoză parazitară.

Cuvinte cheie: *Trichinella spp.,* prevalență, distribuție larvară, vulpea roșie

Nematodes of the genus *Trichinella* are cosmopolitan zoonotic parasites and are among the most widespread parasites of domestic and wild omnivores and predatory animals (3). The risk of infection with *Trichinella spp.* is a major concern in Romania, due to local eating habits (2).

Historically, human trichinellosis was caused by *Trichinella spiralis* and transmitted to humans by consumption of undercooked domestic pork. Today, most cases of trichinellosis are caused by other *Trichinella* species and transmitted by consumption of raw or undercooked wild game meats (5).

Although trichinellosis surveillance systems and laws regulating commercial pork production have limited *T. spiralis* - caused trichinellosis in Europe and the United States, trichinellosis due to consumption of raw and undercooked wild boar and feral hog meat continues to occur throughout Southeast Asia.

Trichinellosis due to consumption of raw or undercooked meats of other infected game, such as bear, deer, moose, and walrus, continues to occur worldwide (5). Only adherence to hygienic practices when preparing wild game meats and cooking wild game meats to recommended internal temperatures can prevent transmission of trichinellosis to humans. Wilderness medicine clinicians should be prepared to advise hunters and the public on the risks of game meat-linked trichinellosis and on how to diagnose and treat trichinellosis to prevent fatal complications (5).

Trichinella nematodes still circulate in various hosts in both domestic and sylvatic environments. Recently, in Europe, the transmission of *Trichinella spp.* to humans has been attributed more to wild animals than to domestic animals. However, domestic animals could still be a source of human infections in some regions (1). For a long period, human trichinellosis represented serious public health and ecological concern because of the social, economic, cultural, and geographic conditions of special areas in our country (12).

Neghina R. et al. (12) and Marincu I. et al. (11) brought new epidemiological and clinical data on hu-

1) Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania", Timișoara, Romania
2)"Victor Babeș" University of Medicine and Pharmacy, Timișoara
*) *Corresponding author: viorel.herman@fmvt.ro*

man trichinellosis from an endemic former industrial area characterized by high rates of poverty and unemployment and from the Western Romanian region after Romania has been reported the highest incidence of human trichinellosis in the world in 2004.

In accordance with the results, the authors of those studies have proposed new and efficient public health strategies which involved the improvement of sanitary conditions, implementation of a more reliable collaboration between veterinarians and pig breeders/consumers, education of the population as well as more careful supervision of the public health services to limit the occurrence of the important zoonotic disease.

MATERIALS AND METHODS

Muscle samples were collected from 32 red foxes hunted over a period of 3 years (2018-2020) and from hunting grounds in Timis County.

The trichinelloscopy and artificial digestion allowed us to confirm the presence of *Trichinella spp.* larvae in the muscles and to evaluate the larval burden on muscle groups.

The muscles examined were:

- Intercostal muscles
- Diaphragm
- Tongue muscles
- Muscles of the right / left thoracic limb
- Neck muscles (cutaneous neck muscles / dorsal and ventral cervical muscles)
- Head muscles (m. temporalis / m. masseter)
- Spine muscles (m. great dorsal / m. thoracic trapezius)
- Muscles on the left / right pelvic limb

RESULTS AND DISCUSSION

The results obtained after trichinelloscopy (Fig. 1, 2) and artificial digestion revealed a prevalence of 28.12% (9/32) of trichinelosis in foxes from hunting grounds in Timis County and examined for three years.

In our country, Borza C. et al. (4) reported for the first time data regarding trichinellosis in sacrificed animals (domestic pigs and wild boars) inspected during the period from January 1998 to November 2011 in the largest of the Romanian counties. The results indicated that 681 domestic pigs (0.01%) on farms out of 5.586.431 and 407 pigs in private households (0.07%) out of 609.325 were found to be infected with *Trichinella*. The *Trichinella* infection was detected in only 4 wild boars (0.5%). This study warns that human outbreaks continue to occur yearly, and the main factors for these include improper handling by the population.

Trichinellosis remains one of the most important zoonotic diseases in Romania. A study conducted by Blaga R. et al. (2) identified *Trichinella spp.* circulating among wild and domestic animals in Romania. The highest prevalence of *Trichinella* infection was detected in wolves (31%), in European wild cats (14%), and in red foxes (7%).

In Poland, the trichinellosis prevalence was 4% in red foxes with 4.09 lpg - mean larval burden (1), while in the northern Canadian communities the Arctic foxes serve as sentinel hosts of *Trichinella spp.,* confirming the presence of the parasite in wildlife in the region. Moreover, larvae of *Trichinella spp.* were recovered from the left forelimb muscle with the highest-burden compared with other body regions (13).

Intensification of *Trichinella spp.* larvae present in the muscle groups of the 9 infested foxes revealed the following mean larval distribution:

- Tongue - 59.10 larvae / gram
- Diaphragm - 14.10 larvae / gram
- Intercostal muscles - 12.80 larvae / gram
- Neck muscles - 9.21 larvae / gram
- Masseter - 12.80 larvae / gram
- Temporalis muscles - 5.25 larvae / gram

Muscles of the right thoracic limb

- Arm muscles - 11.32 larvae / gram
- Forearm muscles - 13.67 larvae / gram
- Shoulder muscles - 11.07 larvae / gram

Muscles of the left thoracic limb

- Arm muscles - 16.92 larvae / gram
- Forearm muscles - 7.25 larvae / gram
- Shoulder muscles - 7.03 larvae / gram

Muscles of the right pelvic limb

- Leg muscles - 9.14 larvae / gram
- Thigh muscles - 9.14 larvae / gram
- Buttock muscles - 9.60 larvae / gram

Muscles of the left pelvic limb

- Leg muscles - 12.35 larvae / gram
- Thigh muscles - 5.82 larvae / gram
- Buttock muscles - 7.82 larvae / gram

The results reveal that the highest larval burden is in the tongue (59.10 larvae / gram) followed by the diaphragm (14.10 larvae / gram) and the arm muscles, respectively the forearm muscles (16.92 larvae / gram; 13.67 larvae / gram). A similar number of larvae were identified in the intercostal muscles (12.80 larvae / gram), masseters (12.80 larvae / gram) and calf muscles (12.35 larvae / gram).

Surprising is the number of larvae identified in the muscles of the thoracic limb, with insignificant differences between the right limb (36.06 larvae / gram) and the left one (31.20 larvae / gram). The same insignificant difference in larval load was identified between the muscles of the right and left pelvic limb (25.99 larvae / gram), again in favor of the right one (27.88 larvae / gram).

Only 9 larvae / gram were quantified in the neck

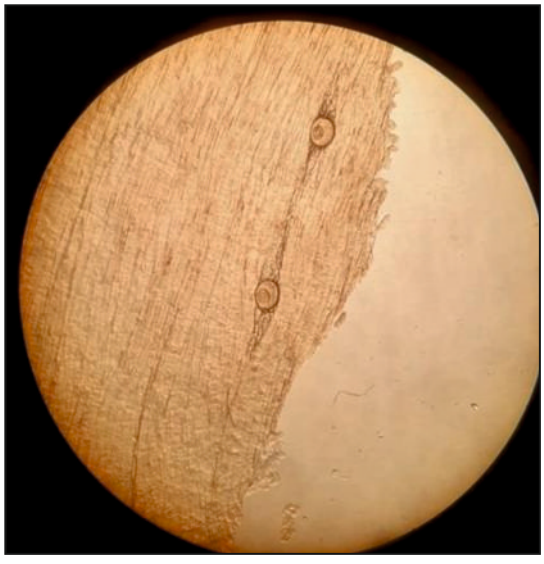

Fig. 1. *Trichinella spp*. – diaphragm

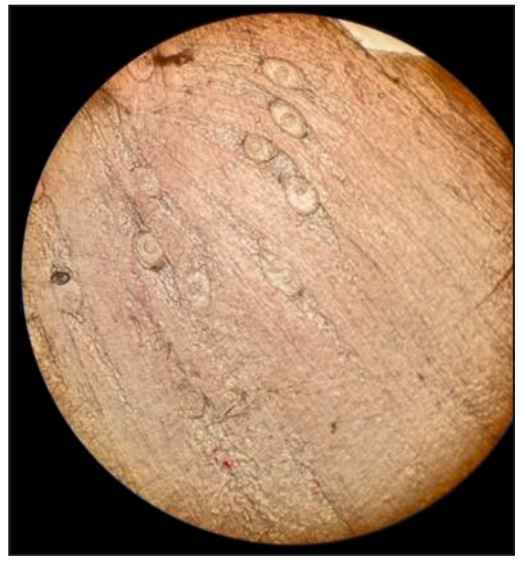

Fig. 2. *Trichinella spp.* – tongue muscle

muscles, while the temporalis muscles showed the lowest larval load (5.25 larvae / gram).

Similarly, results with the present study were been highlighted by Kapel et al. (7) in the studies 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.

A few years later, data obtained from a series of experimental infections carried out by Kapel et al. (8) in pigs, wild boars, foxes and horses revealed the predilection sites of nine well-defined genotypes of *Trichinella*. From all host species, corresponding muscles/ muscle groups were examined by artificial digestion. In foxes, where all *Trichinella* species were 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.

Imre K. et al. (6) provided data about the prevalence of *Trichinella spp.* infections in red foxes (*Vulpes vulpes*) that are hunted in Romanian counties,in which the prevalence of *Trichinella spp.* infection in the backyard and free-ranging pigs range from 0.17 to 2.5%.

The results revealed that the red fox should be considered an important *T. britovi* reservoir in the sylvatic cycle. Of the 25 tested samples, the *Trichinella* larvae from 24 isolates were identified as *T. britovi* (96%), and the larvae from one isolate were identified as *T. spiralis* (4%). The detection of only a single *T. spiralis* - positive isolate suggests that red foxes play a minor role in the epidemiology of the domestic cycle in the investigated area of western Romania.

Artificial digestion of tissue samples performed by Pozio et al. (14) from 60 different muscles from 13 different sites of the infected horse carcass showed that *M. maxillaris, M. hyoideus transversus*, and *M. buccinator* were the 3 most infected muscles. Muscles from the tongue, the masseter, and the diaphragm, which have normally been considered the muscles of choice for diagnosis, were the 4th, 6th and 13th most infected muscles, respectively.

The infectivity of *Trichinella spiralis, T. nativa,* and *T. britovi* was experimentally compared by Kapel et al. (9) in pigs. Surprisingly, for *T. spiralis* and *T. britovi*, the neck muscle appears to be a predilection site in addition to the tongue, the diaphragm, and the jaw. Compared those results with our investigation in red fox, we could emphasize the low ML burden in the neck muscle (9.21 L/gram).

Theodoropoulos G. et al. (16) inoculated 9 isolates of *Trichinella* in a total of 36 sheep. Muscle larvae were found in high numbers in all four sheep inoculated with *T. spiralis*, in lower numbers in two sheep inoculated with *T. pseudospiralis* (USA isolate), and in very low numbers in one sheep inoculated with *T. pseudospiralis* (USSR isolate) and one inoculated with *T. britovi*. In infections of high and moderate larval intensity, predilection sites of *T. spiralis* were the masseter muscles, the tongue, and the diaphragm and those of *T. pseudospiralis* were the masseter muscle and the neck. In low-intensity infections, muscle larvae were detected only in the diaphragm or in pooled muscle samples.

The prevalence of *Trichinella T9* in trapped raccoons (*Procyon lotor*) and several other potential mammalian reservoirs in Hokkaido, Wakayama, and Nagasaki Prefectures were investigated (10). The prevalence and intensity of the muscle larvae were 0.9% and 93.3 larvae/g in raccoons, and 1.6% and 61.6 lar-

vae/g in raccoon dogs, respectively. In raccoons, the muscle density of *Trichinella T9* larvae was highest in the tongue, and larvae were not found in the heart muscle or diaphragm.

Sharma et al. (15) affirm that the wolverine hosts high prevalence (78%), high larval intensity (maximum intensity of infection – 295 larvae per gram), and multiple species of *Trichinella (Trichinella T6, T. nativa,* and *T. chanchalensis)*, likely due to their scavenging habits, apex position in the food chain, and wide home range. Wolverine (especially adult males) should be considered as a sentinel species for surveys for *Trichinella spp.* across their distributional range.

CONCLUSIONS

The identification of *Trichinella larvae* in a percentage of 28.12 of the total number of foxes examined over a period of three years warns of the role that these wild carnivores can play in the epidemiology of this important zoonosis. The muscles of the tongue, diaphragm, masseter and intercostal muscles remain the muscles of choice for performing trichinelloscopy and artificial digestion, although in the fox, an important larval burden has the limb muscles.

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