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Mini Review

IMPORTANT BIOLOGICAL CHARACTERISTICS OF *TRICHINELLA* GENOTYPES

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Abstract

Background. To control *Trichinella*, one of the most important food borne parasites, it is necessary to know the biology of the parasite.

Scope and Approach. The pathogenicity of *Trichinella* genotypes mainly depends on its biological characteristics: reproductive capacity index (RCI), infectivity towards different animal species and humans, differences in the number of newborn larvae (NBL) and susceptibility to environmental conditions.

Key Findings and Conclusions. Among the *Trichinella*, *T. spiralis* has the highest RCI for domestic, synanthropic and some wild animals. This genotype is the most infectious for domestic and wild pigs and has the highest NBL index. *T. spiralis* is more susceptible to high temperatures, freezing and decay of dead host tissues than the sylvatic genotypes. The pathogenicity of *T. spiralis* results in its easy and rapid spread across populations of domestic pigs, thus creating opportunities for human disease. *T. britovi* has a moderate NBL and low RCI for pigs, mice and foxes, but this genotype is resistant to freezing. Other genotypes have high to moderate RCI, NBL and infectivity towards wild animals, have good tolerance towards adverse environmental conditions of low temperature and high humidity, and they usually spread in wild animal populations.

Key words: *Trichinella*, RCI, NBL, infectivity, susceptibility

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INTRODUCTION

Trichinellosis is a parasitic zoonosis caused by nematodes of the genus *Trichinella,* the largest known intracellular parasite. This parasitic nematode is globally distributed and has so far been isolated from mammals, birds and reptiles in the continents worldwide except Antarctica (Pozio, 2016). Human infections have been officially reported in 55 countries. Morbidity and hospitalization rates as well as reported lethal outcomes strongly inform on the severity of the disease among humans. In some countries, *Trichinella* is considered as one of the most important zoonotic food borne parasites (Bouwknegt et al., 2016; Petrović et al., 2017). Today, 12 genotypes are recognized (Korhonen et al., 2016), but *T. spiralis* and *T. britovi* are the only genotypes isolated in Serbia (Petrović et al., 2019). To control trichinellosis, it is necessary to understand the biology and factors influencing the pathogenicity of this parasite.

The pathogenicity of the genus *Trichinella* is associated with the ability of this intracellular parasite to invade host cells without killing them and to reprogram the host cell metabolism to satisfy all the requirements of the parasite. Pathogenicity is influenced by capsule formation, which along with anaerobic metabolism favors the parasite survival in decaying host tissue and protects it from freezing, drying or proteolytic processes (Wu et al., 2013). *Trichinella* pathogenicity varies according to genotype, and is associated with different biological characteristics: reproductive capacity index (RCI), infective potential towards animal species including humans, differences in the number of newborn larvae and susceptibility to environmental conditions (Pozio et al., 1992).

REPRODUCTIVE CAPACITY INDEX

RCI is defined as the ratio of the total number of larvae recovered from the muscle to the number of larvae administered during artificial infection (Kociecka et al., 2003). RCI indicates not only the susceptibility of the host towards a specific *Trichinella* genotype but also the genotype's biological characteristics. According to the RCI values in Table 1, rat, fox and mouse are the most suitable hosts for *T. spiralis.* Rats are also susceptible to *T. pseudospiralis,* whereas the RCIs indicate that synanthropic rats cannot be reservoirs for other genotypes. Pigs are the most susceptible to *T. spiralis* and to *T. britovi*, *T. pseudospiralis*, *T. nelsoni* and *T. zimbabwensis*, but they are not susceptible to *T. nativa*, *T. murrelli*, *T*6 or *T. patagoniensis*. RCI is also influenced by the number of ingested infective larvae and exposure of the infected carcass to environmental conditions such as temperature, relative humidity, whether the carcass was lying under snow cover, etc. (Rossi et al., 2019).

Table 1. Reproductive Capacity Index of *Trichinella* species/genotypes (Pozio et al., 1992; Murell et al., 2000; Hill et al., 2005; Krivokapich et al., 2012; Sadaow et al., 2013)

Species	Pig	Mouse	Rat	Wild rodents	Fox
T. spiralis	80-89	188-315	185-237	33-99	154
T. nativa	<1	24-74	0.02-1	/	40
T. britovi	8-10	11	0.02-1	/	7
T. pseudospiralis	8-10	20-30	47-62	/	17-59
T. murrelli	<1	1-9	0.7-2	30-160	107
Trichinella T6	<1	0.4-38	0.1	/	8
T. nelsoni	8-10	3-31	0.6	/	61
Trichinella T8	/	/	0.5	/	/
Trichinella T9	/	/	/	/	/
T. papuae	/	28-56	6-11	241	/
T. zimbabwensis	4-8	9	6	/	/
T. patagoniensis	<1	36-94	<1	/	/

/-no data available

INFECTIVITY

Infectivity is the ability of a pathogen to produce an infection. The infectivity of *Trichinella* genotypes is assessed according to the number of larvae that cause infection (> 1 lpg – larvae per gram of muscle) (Hill et al., 2005). *T. spiralis* is considered the most infective *Trichinella* for humans, domestic and wild pigs and herbivores (horses, cattle, sheep and goats) (Table 2). *T. britovi* produce a smaller number of muscle larvae in domestic pigs, and these larvae are relatively rapidly eliminated by the immune system of the host; however, *T. britovi* can induce outbreaks in humans consuming infected pork meat (Kurdova et al., 2004; Krivokapich et al., 2019). Sylvatic carnivores are considered the most suitable hosts for the majority of *Trichinella* genotypes (Pozio, 2016). *T. nativa, T. murrelli* and *T6* have very low infectivity towards domestic pigs. *T. nativa* is rarely detected in naturally infected wild boars and occurs predominantly in immunocompromised animals living in extremely poor conditions (Nockler et al. 2005). *T. nativa* and *T6* are 10,000 times less infective towards pigs than *T. spiralis* (Hill et al., 2009). Artificial infection with *T6, T. zimbabwensis* and *T. patagoniensis* revealed the low infectivity of these *Trichinella* for pigs, as these pathogens persist in pork meat only for a short period (Kapel and Gamble, 2000; Pozio and Zarlenga, 2019). *T. nativa* persists for one month in infected 3-month-old piglets, whereas isolation of muscle larvae from 1-year-old pigs is not possible even after artificial infection with large numbers of larvae (10,000) (Hill et al. 2009). In adult pigs infected with *T. nativa* or *T. murrelli*, antibody titers can be recorded over a period of 3-5 weeks post infection, but this is without detectable muscle larvae, indicating the infection is overpowered by the host immune system (Kapel and Gamble, 2000).

Table 2. Infectivity of Trichinella towards various animal species (Theodoropoulos et al., 2000; Kapel and Gamble, 2000; Krivokapich et al., 2012)

Genotype	Pig	Mouse	Rat	Wild boar	Fox	Sheep	Horse	Birds
T. spiralis	+++	+++	+++	+++	+++	+++	+++	-
T. nativa	+	++	+	+	+++	-	/	-
T. britovi	++	++	++	++	+++	+	++	-
T. pseudospiralis	++	++	++	+/++	+++	+	/	+++
T. murrelli	+	+/++	+/++	+	+++	-	++	-
Trichinella T6	+	++	+	+	+++	-	/	-
T. nelsoni	++	++	++	++	+++	-	/	-
Trichinella T8	/	/	+	/	/	/	/	/
Trichinella T9	/	/	/	/	/	/	/	/
T. papuae	++	+	+	++	+	/	/	-
T. zimbabwensis	+	+	+	/	/	/	/	/
T. patagoniensis	+	++	+	/	/	/	/	/

+ weak infectivity; ++ moderate infectivity; +++ high infectivity; /-no data available

The incidence of *T. spiralis* in domestic pigs is 1.9 times higher than that of *T. britovi* and 58.5 times higher than that of *T. pseudospiralis*. In carnivores, the incidence rates of *T. britovi* are 8.0 and 107.4 times higher than those of *T. spiralis* and *T. pseudospiralis*, respectively. All other *Trichinella* genotypes occur only in carnivores with the few exceptions pertaining to *T. nativa* and *T. nelsoni* (Pozio and Zarlenga, 2019). Compared to *T. spiralis, T. patagoniensis* is 4 and 2,200 times less infective for mice and rats, respectively (Krivokapich et al., 2012).

PRODUCTION OF NEWBORN LARVAE AND CAPSULE FORMATION

If compared with other genotypes, *T. spiralis* is characterized by the highest production of newborn larvae (NBL), as the parasite produces 110 NBL during the first three days of life. The lowest 3-day NBL rates were recorded in *T. nativa, T. murrelli, T*8 and *T. patagoniensis* (29.8, 30.6, 26.0 and 25.9, respectively), whereas other investigated genotypes demonstrated moderate NBL production. Capsule formation occurs most rapidly in *T. spiralis* (day 16 post-infection), while other encapsulating genotypes develop capsules from day 18 to day 34 post-infection. The time required for capsule formation varies according to host species – in rats, the process is completed in 30-60 days after infection onset, although the process can extend to over 4 months for *T. murrelli* encapsulation (Pozio et al., 1992; Krivokapich et al., 2012).

SUSCEPTIBILITY TO ENVIRONMENTAL CONDITIONS

T. spiralis is far more susceptible to high temperatures, freezing and decay of dead host tissues than the sylvatic genotypes. The rare occurrence of this species in sylvatic cycles is likely due to the pathogen's susceptibility to environmental conditions. Freezing at -20°C and drying at 60°C for 24h inactivate *T. spiralis* larvae in pork meat. Only *T. britovi, T. nativa* and *T*6 are resistant to freezing, so they can survive in frozen muscle and continue their natural life cycles if ingested by a suitable host. *T. nativa* is considered the most resistant genotype, which was identified during an epidemic outbreak of trichinellosis in Alaska after consumption of bear meat previously frozen for 81 days at -18°C (Kumar et al., 1990). Freeze-resistant genotypes manifest longer survival periods in carnivore meat than in pork and rat meat. Muscle larvae of *Trichinella* of medium age (10 to 20 weeks post-infection) have better tolerance to freezing than young or old larvae (5 and 40 weeks post-infection, respectively). Environmental conditions play an important role – high humidity increases the resistance duration, as do the duration and temperature of freezing (optimal temperature is around -20°C). In Arctic and subarctic regions, the freeze tolerance of *T. nativa* and *T*6 compensates for their relatively low infective potential (Kapel and Gamble, 2000; Pozio 2016; Pozio and Zarlenga 2019).

Part of the physiological mechanism of *Trichinella* survival includes the ability of muscle larvae to survive in decaying carcasses. Longer muscle survival durations are inevitably associated with an increased probability of the carcass meat being consumed by scavenging animals. Anaerobic larval metabolism in muscle cells enables survival in decomposing tissues. Environmental conditions such as high humidity and low temperatures favor the survival of the larvae even in conditions of complete exposure of the tissues to the conditions. Although *Trichinella* genotypes that do not form capsules can survive in carcasses, the encapsulating genotypes can survive longer. The high carbohydrate content of the capsule is considered to have protective effects. Muscle larvae of *T. nativa, T. britovi* and *T*6 can survive freezing for several months or even years (Lacour et al., 2013). Non-encapsulated *T. papuae* with preserved infectivity can survive in decaying tissues for 9 days at 20-24°C and 40 days at 5°C (Owen and Reid 2007; Pozio and Zarlenga 2019). Survival durations with maintained infectivity in extremely decayed meat are 4 months for *T. spiralis* and 2 months for *T. britovi* and *T. nelsoni*. *T. zimbabwensis* survives 4 weeks at -5°C in fox carcasses (Pozio, 2016). It is important to emphasize that in spite of the high freeze-susceptibility of *T. spiralis*, processing meat in which the presence of any of the *Trichinella* genotypes has been confirmed is prohibited in Serbia (and in EU countries). Thus, the carcasses of infected animals must be adequately disposed of in a manner that prevents further spread of this parasite (Urošević et al., 2013).

The pathogens' adaptive capacity to environmental conditions is of vital importance for the life cycle and spread of *T. nativa, T. britovi, T*6, *T. murrelli* and *T. patagoniensis*. In other *Trichinella* genotypes, the pathogen's adaptation to the host immune response plays a much more important role. Accordingly, *T. spiralis* predominantly occurs in pigs

and rats in diverse habitats (Pozio and Zarlenga 2019). Animals that acquire *Trichinella* in their natural habitats usually have smaller numbers of larvae in the predilection sites (<10 lpg) (Nockler et al., 2006); however, some of our research has revealed highly-infected animals (Petrović et al., 2012). If the next host becomes infected with a small number of larvae, this could provoke a weak immune response and consequent high risk for recurrent infection. In that respect, it is not uncommon that muscle larvae of different ages or of two different *Trichinella* genotypes can be found in the same host (Sharma et al., 2019).

CONCLUSION

The biological characteristics of the different *Trichinella* genotypes affect their spread in domestic and wild animal populations. The most commonly isolated *Trichinella* genotype, *T. spiralis,* spreads easily and rapidly across populations of domestic pigs thanks to its high RCI, NBL and infectivity. *T. britovi* has moderate NBLs and low RCIs for pigs, mice and foxes and very low RCIs for rats and wild rodents. However, its resistance to freezing enhances the spread of this genotype in sylvatic animals. Other *Trichinella* genotypes have moderate to high RCIs, NBLs and infectivities towards wild animals, but good resistance to freezing, and they usually spread in wild animal populations.

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Competing interests

There is no competing interests

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ZNAČAJNE BIOLOŠKE KARATKERISTIKE GENOTIPOVA TRIHINELA

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Kratak sadržaj

Uvod. Za kontrolu trihinela, jednog od najvažnijih parazita koji se prenose hranom, neophodno je poznavati biologiju parazita.

Cilj i pristup. Patogenost različitih genotipova *Trichinella* u najvećoj meri zavisi od njihovih bioloških karakteristika: indeksa reproduktivnog kapaciteta (RCI), infektivnosti prema različitim vrstama životinja i ljudima, razlika u broju novorođenih larvi (NBL) i otpornosti prema uslovima spoljašnje sredine.

Ključni nalazi i zaključak. *T. spiralis* ima najviši RCI prema domaćim, sinantropnim i nekim divljim životinjama. Ovaj genotip je najinfektivniji za domaće i divlje svinje, takođe ima najviši NBL. *T. spiralis* je osetljivija prema visokim temperaturama, zamrzavanju i procesima raspadanja u tkivima uginulih životinja u poređenju sa silvatičnim genotipovima. Patogenost *T. spiralis* je dovela do brzog i jednostavnog širenja u populaciji domaćih svinja i posledičnog oboljevanja ljudi. *T. britovi* ima umeren NBL i nisku RCI kod svinja, miševa i lisica ali ovaj genotip je otporan na zamrzavanje. Ostali genotipovi imaju visok ili umereno visok RCI, NBL i infektivnost prema divljim životinjama sa dobrom otpornosti prema uslovima spoljašnje sredine i uglavnom se šire u populaciji divljih životinja.

Ključne reči: *Trichinella*, RCI, NBL, infektvnost, osetljivost