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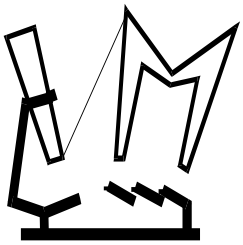

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MOLECULAR DIAGNOSTICS OF TRICHINELLA SPECIES: NEW DATA ON TRICHINELLA LIFE CYCLE IN VOJVODINA REGION

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Abstract – Trichinellosis is a zoonosis caused by a parasitic larvae of genus *Trichinella*. Serbia belongs to a group of countries where *Trichinella* is present in domestic animals, but also in synanthropic and sylvatic animals. The research of trichinellosis that has been carried out in Serbia so far, aimed at reducing the risk of transmission of trichinellosis on people and reducing economic loses in pig production, but sylvatic trichinellosis has been poorly researched. The aim of this study is molecular determination of *Trichinella* larvae isolated from domestic, synanthrophic and sylvatic animals to determine the specificity of *Trichinella* life cycle in Vojvodina region.Totaly of 470 samples were examined, trichinella were isolated from 14 samples and examined by molecular methods, only one species was determined - *T. spiralis*. The given data point out that in the implementation of the measures for reducing the trichinellosis in domestic animal is necessary to include measures for prevention of the transmission of trichinellosis from domestic pigs to sylvatic animals.

Key words – *Trichinella*, domestic and sylvatic animals.

I. INTRODUCTION

Trichinellosis is a zoonosis caused by a parasitic larvae of genus *Trichinella*, it is an endemic disease which is present in most of European countries. In Serbia the endemic regions for trichinellosis are Srem, the valleys of the Danube, Drina and Kolubara [5]. The domestic pig is the main reservoir of trichinellosis for humans, infection usually appears after consumption of smoked meat pruducts where live worms are present [20]. Serbia belongs to a group of countries where *T. spiralis* is present in domestic but also in sylvatic and synanthrophic animals. Synanthrophic animals are species of wild animals that live near and have benefit from humans, some species of rodents, foxes, jackals, pigeons [16].

Until 1972. year the only known *Trichinella* species was *T. spiralis*. In last thirty years eight *Trichinella* species were discovered*: T. spiralis, T. britovi, T. nativa, T. pseudospiralis, T. papuae, T. nelsoni, T. murrelli, T. zimbabwensis* and three genotypes which are not classified yet: Trichina T6, Trichina T8 i Trichina T9 [10, 9]. Conventional microscopy methods for meat inspection can not determine the type of *Trichinella* because there are no morphological differences between the *Trichinella* species. *Trichinellas* differ in their characteristics: the ability to form capsules, the resistance to freezing, the production of newborn larvae. The biggest difference occurs in the susceptibility of certain species to different types of *Trichinella*.

T. spiralis is most frequently present in domestic and wild pigs, compared to other types of *Trichinella* it is the most pathogenic for humans. It is widespread around the world. *T. spiralis* is the only *Trichinella* species which is highly infective for domestic pigs, rats and mice. It is also infective for sylvatic animals, wild pigs, bears, rodents, foxes and jackals. Comparing to other species, *T. spiralis* has the highest production of newborn larvae *in vitro* and is the least resistant to freezing [14, 16, 9]. In Europe, wildlife represents the most important reservoir of *Trichinella*, which makes eradication impossible and explains why the parasite continue to circulate, even though the prevalence in wildlife can be very low for many years [17].

T. britovi is present in wild animals. The second most spread trichinella species in humans, it is infectious to sylvatic carnivores, sylvatic omnivorous, domestic pigs, horses and humans. Regarding biological characteristics it is very

similar to *T. spiralis* and can be distinguished by low infectivity to rats, greater resistance to freezing and moderate infectivity for swine. *T. britovi* is transmitted primarily among sylvatic hosts, probably due to low reproductive capacity in pigs and *synanthropic rodents*. The first cases in Serbia were confirmed in 2011 by Cvetković *et al*. [4]. It is also present in the neighboring countries: Bulgaria, Romania, Croatia, Italy and Macedonia [9].

T. pseudospiralis is slightly smaller than other species. In addition to *T. papuae,* it is the only type of *Trichinella* that does not form a capsule. *T. pseudospiralis* is infectious to birds and mammals, has low reproductive capacity in the body of rats and moderate in pig. It may be rarely found in humans [14, 16, 4].

T. nativa may be found in in sylvatic animals which live in cold areas. It has been reported in the mammals in the reagion of the Baltic Sea. The findings of *T. nativa* are not expected in the game in Serbia, however it is important to note that this type of *Trichinella* is resistant to freezing [7, 9].

By the applicaton of molecular techniques it is possible to obtain specific genetic information from the minimal sample volume and thus a reliable way to determine the species of *Trichinella*. The aim of this study molecular determination of *Trichinella* larvae isolated from domestic, synanthrophic and sylvatic animals to determine the specificity of *Trichinella* life cycle in Vojvodina region.

II. MATERIALS AND METHODS

Samples were collected in hunting grounds in Vojvodina from october 2012. year until february 2013.year. Samples originating from domestic pigs were meat products collected by veterinary officers during trichinella outbreaks. Samples originating from sylvatic animals were diaphragms. Totaly of 470 samples were examined: meat products from domestic pigs 34, wild pigs 377, foxes 21 and jackals 38. Presence of *Trichinella* larvae were examined by artificial digestion according to Commision Regulation (EC) No 2075/2005 [3] 14 samples were positive, larvae were collected and examined, DNA were isolated by standard phenol-chlorophorom method of extraction withe usage of proteinase K [18]. Determination of isolated muscle larvae was made by PCR method [1], for primer sets were used, which enable diferentiation of species and genotype in *Trichinella* genus [21].

III. RESULTS AND DISCUSSION

1. Trichinellosis in humans in Vojvodina

According to Ofori-Belić *et al.* [12] the occurrence of human trichinellosis in Serbia showes a strong seasonality ($P < 0.001$), most of the cases happenes at winter. The incubation period ranged between one and 33 days. The mean time between onset of symptoms and admission was nine days. Family outbreaks were the most frequent. Smoked pork products were the dominant source of infection (76%). Fever was the most frequent clinical manifestation (90%), followed by myalgia (80%) and periorbital edema (76%).

In our investigations [20] in table 1, outbreaks from the nine years period between 2002. and 2011. are presented, 983 humans become infected with *Trichinella* species in Vojvodina region. There is difference in gender of ill people: 56.45% of sick people were male and 43,54% were female. The highest number of ill humans was detected in year 2005. - 277 persons and in year 2002. - 275 persons. In these years three people died from trichinellosis.

Table 1 Human trichinellosis in Vojvodina, from 2002. to 2011. year [20]

Humans	Age of ill pepole – no of ill pepole								
age	< 6	7-14	15-19	20-29	30-39	40-49	50-59	>60	Total
male	17	64	37	92	101	111	69	64	555
Female	23	29	25	64	71	84	74	58	428
Total	40	93	62	156	172	195	143	122	983
%	4,06	9,46	6,31	15,87	17,50	19,83	14,55	12,41	100,00

Human trichinellosis is the most common in age between 30 to 49 years (37,33% of all ill people), it is rare in small children, under six

years (4.06%). Source of infection usually are traditional meat products which are not cooked, only salted and smoked. Eating habits directly have influence on gender and age distribution of ill humans. These traditional products which aree strong tasted and salty, mostly like to eat adult males. There was no correlation between the prevalence of infestation among swine and the frequency of human outbreaks in the corresponding districts. According to the data obtained from veterinary and medical authorities, Sofronić-Milosavljević *et al* [19] presumed that the absence of human disease in the above mentioned districts reflects the effectiveness of control measures and public education conducted in the field. Wild pigs are the source of infestation for humans, several outbreaks happened after consumtion of game meat, in Serbian legislative is obligatory to control game meat for *Trichinella* presence.

2. The life cycle of *Trichinella*

The results of *Trichinella* detection and determination are presented in table 2.

Two ways of maintaining and transmitting the *Trichinella* parasite are distinguished. One refers to the cycle in domestic animals, and the other to sylvatic cycle. It is questionable whether sylvatic cycle is independent from the cycle in domestic animals, and to which extent the game is a reservoir for *Trichinella*. The main indicators of sylvatic cycle are: prevalence of trichinellosis in different wild animals species, the degree of infestation and species of *Trichinella*. In sylvatic cycle, transmission of the parasite primarily occurs among carnivores (foxes, wolves, jackals), and to a lesser extent among omnivorous (wild boar, bears and rats). The natural habitat and its characteristics have main influence on the life cycle of *Trichinella*. Climatic conditions in Vojvodina are favorable to the life cycle of *T. spiralis*, the prevalence of this species of *Trichinella* is very small in cold and tropical areas. Vojvodina is a flat region with no geographical barriers which clearly separate the sylvatic habitat from the habitat of domestic animals, as is the case in mountainous areas.

3. The sylvatic cycle in Vojvodini

In our examinations (Table 1) trichinellosis has been found in wild boars, foxes and jackals. In other parts of Serbia, it was found also in raccoons, wolves, and bears [4].

Table 2 Presence of *Trichinella* species in samples from domestic and sylvatic animals from Vojvodina region

Animal species	No of samples	Positive (%)	Trichinella species	Maximum no of larvae per g
Domestic swine *Sus scrofa*	34	8 (23.53%)	*T. spiralis*	570 larvi/g
Wild swine *Sus scrofa*	377	2 (0.53%)	*T. spiralis*	1100larvi/g
Red fox *Vulpes vulpes*	21	1 (4.76%)	*T. spiralis*	1 larva/g
Golden jackal *Canisa aureus*	38	3 (7.89%)	*T. spiralis*	3 larve/g
Total	**470**	**14 (2.98%)**	*T. spiralis*	-

Regardless of the etiological agents and geographic region, the main reservoir of sylvatic *Trichinella* are carnivorous with cannibalistic and scavening behavior [2]. So far, red fox was the main reservoir of sylvatic *Trichinella* in this area, however, the increasingly important role of jackals must be pointed out. The presence of jackals in Serbia has been evident for last twenty years. Coming over the Carpathian Mountain and across the Danube basin, the jackals first settled in eastern Serbia, and later on expanded to Belgrade and on the territory of Vojvodina. Today the jackal population is large. They inhabit different terrains and can be found in the lower mountains and on open hunting plains. The jackals are usually caught in the wild boar and other game hunting. The increase in jackal population resulted in reducing the deer and fox population.

Our studies determined a relatively high prevalence of *Trichinella* in jackals (7.89%), foxes (4.76%) and boars (0.53%) on the territory of Vojvodina. In eastern Serbia higher prevalence were found jackals(53.8%), foxes (12.3%) and boars (11.7%) [22]. In the countries where trichinellosis of domestic animals has been eradicated, such as Denmark, the prevalence of sylvatic trichinellosis is very low (0.001%) [6]. The degree of infestation in omnivore and carnivore game in our country is higher (30 larvae/10g) comparing to the countries without trichinellosis in domestic animals, such as Denmark (1 larvae/10g). Our investigations have revealed that in wild boar infestation is very high and is 1100 larva/g. If the prevalence sylvatic trichinellosis on a particular geographical area is high, then the risk of the spread of infestations to domestic pigs is significant, especially in the grazing habitat.

4. Trichinella species detected in sylvatic and domestic animals in Vojvodina

In the Vojvodina region only *T. spiralis* has been proved –Table 2, while in Serbia *T. spiralis* and *T. britovi* have been detected in wild animals [4, 22]. According to Živojinovic *et al* [22] in eastern regions of Serbia, investigations performed during the 2009-2010 period, *T. britovi* was identified in 31% of isolates from wildlife of the Braničevo district and *T. spiralis* was found in 53% of wild animals; mixed infections were observed in 16% of the animals examined. The presence of *T. spiralis* in wild animals is related to the *Trichinella* in domestic animals. Murell et al [8] proved that jackals, foxes, rats and other synanthropic animals are a link between sylvatic and domestic animals trichinellosis if the infestation is caused by *T. spiralis*. The incidence of *T. spiralis* is directly affected by the spatial proximity of habitat in which wild and domestic animals coexist. *T. spiralis* is rarely found in wild animals that live far away from villages and farms. Sylvatic *Trichinella*, such as *T. britovi*, can be found in domestic animals. However, this type of infestation presents the end of a life cycle because sylvatic *Trichinella* can be maintained only within sylvatic population of carnivores that live in natural habitat [14]. The main factor responsible for the occurrence of *T. spiralis* in wild carnivores are their eating habits. Animals with cannibalistic and scavening behavior in sylvatic habitat present the pathway of spreading of sylvatic trichinellosis. However, in areas such as Vojvodina, where jackals and foxes live near human settlements and have the access to the remainings of domestic animals, the risk of infestation with *T. spiralis* is increased.

Wild pigs present an important reservoir for the spread of *Trichinella* and are a direct source of infestation for humans. Very high infestation in wild pigs is not uncommon, like it was found in our examinations (Table 2) and in examinations of other authors [11]. In Serbia several cases of human trichinellosis were reported after consumption of wild boar meat. According to our studies only *T. spiralis* was found in wild boars (table 2). Besides domestic pigs, wild boars are the species that is most susceptible to this type of *Trichinella*. It is believed that the life cycle of *T. spiralis* may include circulation from domestic pigs to wild boars and vice versa. An important indicatora of epidemiology in wild boars are the behavioral characteristics of this species. Wild boars are very tolerant to the presence of humans and often gaze in the areas that men cultivate. An important source of *Trichinella* for wild pigs and rats are domestic animal waste, which may be found in the areas with inadequate veterinary-sanitary control. The finding of *T. spiralis* in domestic pigs and wild animals hunted near human settlements can be explained by the behavior to raise pigs near rivers (e.g., Danube), small waterways and ponds where they can be in touch with wildlife. The husbandry conditions on 90% of these backyard farms are very poor due to the intentional feeding of food waste containing pork scraps, scavenging of pigs in garbage dumps, and the improper disposing of pig carcasses in the field [22].

5. Some features of Trichinella cycle in domestic animals in Vojvodina

According to our study [13], examinatons of rats collected in the control measures for the eradication of trichinellosis on one farm in Vojvodina, extremely high prevalence was found (85.71%) with an extremely high degree

of infestation (900 larvae /g). The role of rats in the epidemiology of trichinelosis is the subject of discussion in the scientific community. While some authors consider rats as main reservoir for *Trichinella*, others support the view that they are accidental hosts. *Trichinella spp*. can be found only in rats on farms where already exists pig trichinellosis or in landfills where remains of pigs with trichinellosis are thrown. There are no reports about the presence of *Trichinella* in rats in areas where there is no *Trichinella* in sylvatic or domestic animals. Today it is considered that rat trichinelosis is a major marker of trichinelosis in pigs and that the main reservoir of infection for both species is inadequately removed pigs remains with trichinellosis [15]. Since the *T. Britovi is* rarely found in rats, it is considered that they dont have an important role in the epidemiology of sylvatic trichinelosis [9].

IV. CONCLUSION

The molecular determination of *Trichinella* larvae isolated from domestic, synanthrophic and sylvatic animals gave us some informations about the specificity of *Trichinella* life cycle in Vojvodina region. The *T. spiralis* is dominant *Trichinella* species among domestic and wild animals. This phenomenon is directly affected by cohabitation of domestic and wild animals in the Vojvodina as a result of lowland terrain features. The high prevalence of *Trichinella* in domestic and in wild animals, is primarily affected by human disrespect and disregard of veterinary-sanitary measures.

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