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# **PHD THESIS**

**EPIDEMIOLOGICAL, CLINICAL AND PARACLINICAL FEATURES  
OF TRICHINELLOSIS IN WESTERN ROMANIA**

## **A B S T R A C T**

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## ABSTRACT

Trichinellosis, a zoonosis transmitted through the consumption of raw meat or insufficiently cooked meat, is widespread globally, affects both humans and animals and has been adapted to a wide range of hosts, including domestic animals, wildlife, and humans.

The collection of reliable epidemiological information on animal and human infections is not consistent worldwide. The global distribution of *Trichinella* and the different cultural eating habits are the main factors for human infections in both industrialized and non-industrialized countries. Compared to other foodborne parasitic diseases, overall burden is significantly low although the European region accounted 69% of the global burden according to the World Health Organization.

There has been a decrease in incidence worldwide, but the trend seems to be changing frequently as an European Union report in 2017 found that Romania had the highest number of positive fattening pigs that weren't raised under controlled housing conditions. The European Union has experienced a rare occurrence of pork-related trichinellosis due to modern pig farming. However, the development of extensive outdoor breeding traditions can facilitate the transmission of *Trichinella* from wild animals to pigs.

In countries where veterinary inspection, responsible farm management and appropriate quality-controlled diagnostic methods are practiced, *Trichinella* has been almost eliminated from pigs. In many less developed countries, the prevalence of *Trichinella* infections has increased due to the increase in the number of small farms.

According to ECDC data, Romania reported the majority of confirmed cases of trichinellosis in Europe, up to 2015 and the notification rate, as well as the number of hospitalized cases, has been steadily decreasing at the national level. Moreover, the number of trichinellosis cases in Western Romania outnumbered the number of confirmed cases at the national level in countries such as Belgium, Germany, Latvia, Lithuania, Poland, and Spain. Recently, between 2016-2020, Bulgaria has reported the highest number of confirmed cases since 2016 and Italy since 2020.

This doctoral thesis aimed to identify and investigate epidemiologically, clinically, and para-clinically the hospitalized cases of human trichinellosis in the western region of Romania and to determine the risk factors for this zoonosis. Also, this thesis aimed to investigate the

medical records of patients diagnosed with trichinellosis between January 17, 2010 - December 31, 2020, who were hospitalized in infectious diseases hospitals, located in four counties (Arad, Caraș-Severin, Hunedoara and Timiș) from Western Romania.

The preoccupations for studying the epidemiological, clinical, and paraclinical features of trichinellosis led to the creation of a serum biobank for 1347 consecutive blood donors attending the Regional Blood Transfusion Center in Timișoara, Romania.

To determine the seroprevalence of *Trichinella* infection in the adult population from western Romania is an objective that has not been addressed in the field. Knowledge of seroprevalence is important given that Romania has reported more than half of EU trichinellosis cases in the last decade, as well as the asymptomatic evolution of this zoonosis in people who consume small or moderate amounts of infected meat.

The present thesis is divided into three parts: the general part, the special part and conclusions.

**The general part** includes information regarding the current state of knowledge on trichinellosis including: etiology, life cycle, epidemiology, diagnosis, treatment and prophylaxis.

**The special part** consists of three studies. It includes one observational cohort retrospective study. The second study is across-sectional observational study focused on the seroprevalence and risk factors of *Trichinella spiralis* infection in blood donors from Western Romania. The third study is an evaluation of knowledge of the disease among blood donors from Western Romania.

**The conclusions** include the most important aspects noticed in the three observational studies and the basic recommendations for the prevention and control of trichinellosis.

## Research objectives

This research was conducted following three main objectives:

1. Identification and investigation of the cases diagnosed and hospitalized in clinical departments of infectious diseases hospitals, from Western Romania including (Arad, Caras-Severin, Hunedoara and Timis) counties between 2010-2020.
2. Assessment of the seroprevalence of anti-*Trichinella* antibodies in voluntary blood donors from Western Romania.
3. Evaluation of knowledge of the trichinellosis among blood donors from Western Romania.

## TRICHINELLOSIS IN HOSPITALIZED CHILDREN AND ADULTS FROM WESTERN ROMANIA, 2010-2020

This study evaluated the trichinellosis cases diagnosed in the infectious disease departments of hospitals from Western Romania. Epidemiological, clinical, therapeutic and laboratory aspects of the disease cases were analyzed in hospitalized children and adults.

The databases of infectious disease hospitals located in this region were investigated for the period between 17 January 2010, and 31 December 2020. Based on the patients medical records, 133 cases of trichinellosis were identified. The diagnosis was established taking into account the history of consumption of raw or insufficiently cooked meat, the clinical symptoms, the serological test results for specific *Trichinella* antibodies, the results of the muscle biopsy of the patients and the epidemiological links (exposure to a common source of contaminated meat).

The county with the most patients was Arad with 46.62% (62/133), followed by Hunedoara with 26.32% (35/133), Timiș with 22.56% (30/133) and Caraș-Severin with 4.51% (6/133)(**Figure 1**).

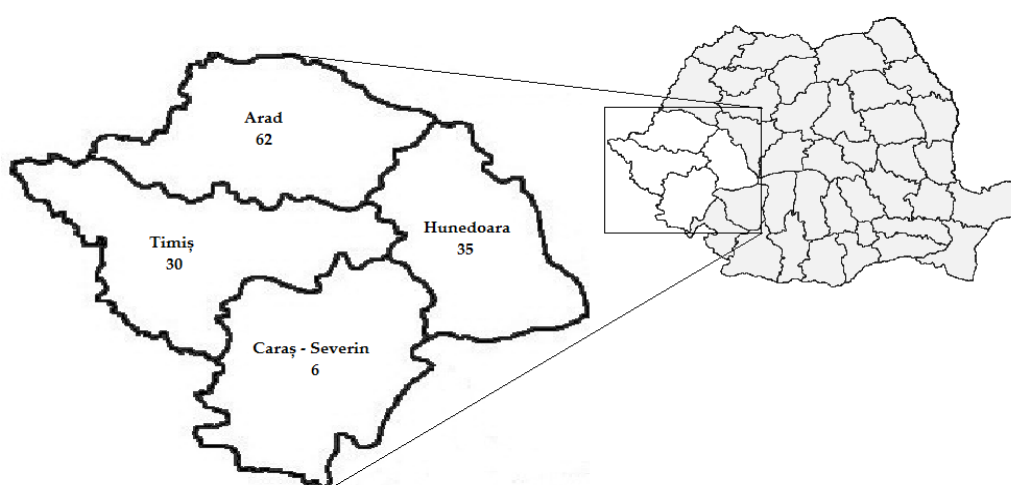


Figure 1. Distribution of trichinellosis cases in Western Romania by county residence, 2010 - 2020.

The highest incidence (number of cases per 100,000 inhabitants) of trichinellosis, between 2010 and 2020, was 14.4 in Arad county, followed by Hunedoara county with 8.36, Timiș county with 4.39 and Caraș-Severin county with 2.03.

The analysis of the results showed a not significant decrease general trend in the incidence of trichinellosis, both in children ( $r=-0.23$ ,  $p=0.48$ ) and adults ( $r=-0.55$ ,  $p=0.07$ )(**Figure 2**).

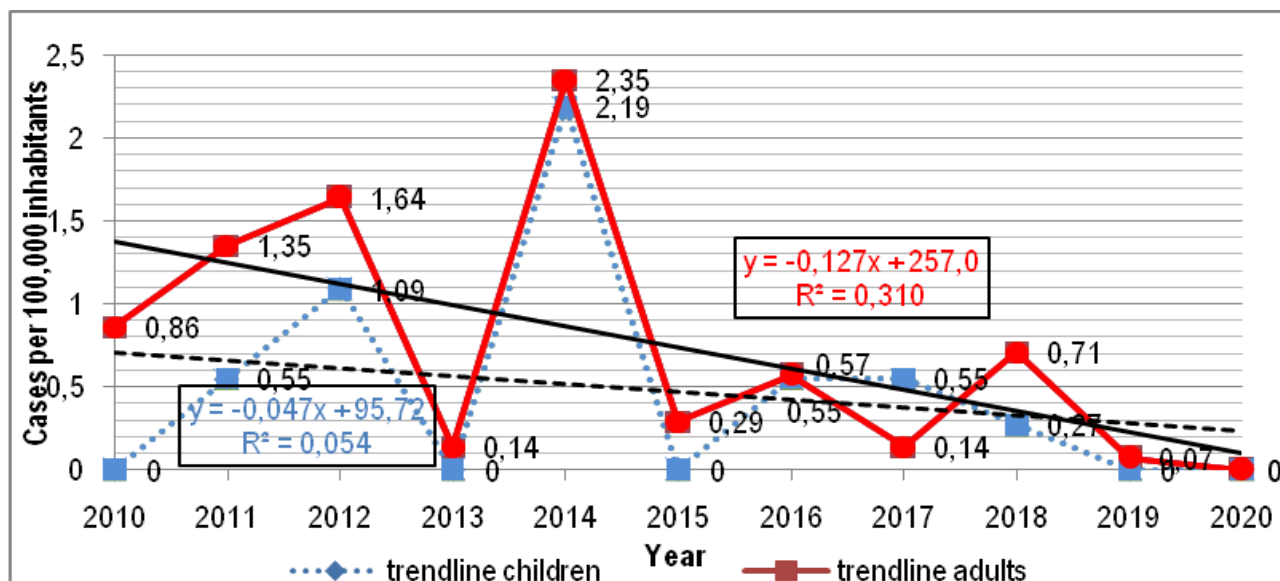


Figure 2. Distribution of trichinellosis cases in children and adults from Western Romania, 2010-2020.

The distribution of trichinellosis regarding season during the studied period, showed that the highest number of cases were registered in winter with 63.91% (85/133) followed by autumn with 28.57% (38/133), spring with 6.02% (8/133) and summer with 1.5% (2/133). The data analysis indicates a not significantly increasing trend of trichinellosis cases ( $r = 0.90$ ,  $p = 0.09$ ) (Figure 3).

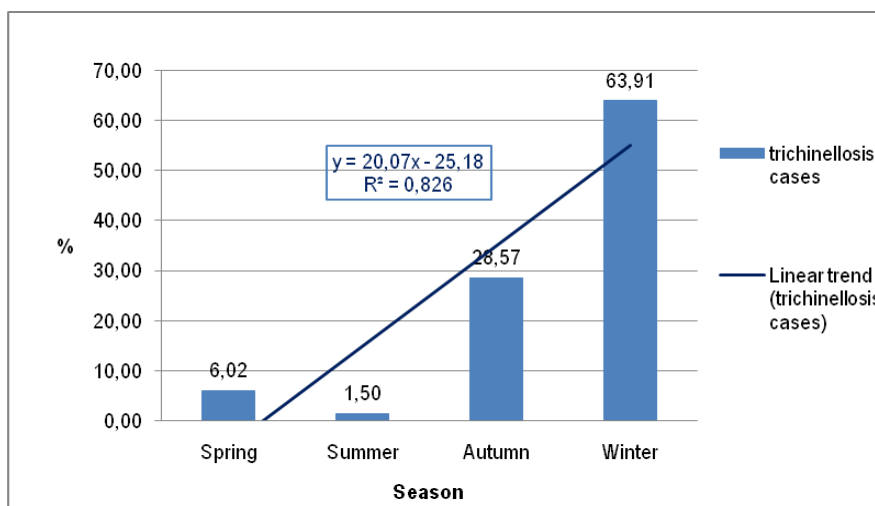


Figure 3. Distribution of trichinellosis cases in Western Romania, 2010-2020

Digestive symptoms were most frequently present in children, in 78.94% (15/19) of cases, fever was present in 57.89% (11/19), eyelid or facial edema was present in 57.89% (11/19), and myalgia was present in 52.63% (10/19). In 87.71% (100/114) of the adults, myalgia was present, fever was present in 77.19% (88/114), digestive symptoms were present in 68.42% (78/114), and eyelid or facial edema was present in 66.66% (76/114). The patients

also presented other symptoms such as headache, asthenia, conjunctival and subungual hemorrhages, skin rashes, chills or excessive sweating. Data analysis showed no significant difference in clinical signs and symptoms between children and adults ( $p=0.31$ ).

The laboratory findings showed that the eosinophil count, leukocyte count, and erythrocyte sedimentation rate were not significantly different between children and adults. Only 23 (17.29%) of the 133 patients diagnosed with trichinellosis were tested for *Trichinella* spp. specific antibodies; 65.22% (15/23) tested positive, without other subsequent serological investigations or molecular tests for *Trichinella* spp. identification.

Albendazole or mebendazole were prescribed to all patients, whether they were children or adults, but those with severe symptoms were given a combination of anthelmintics and corticosteroids. The treatment of choice was albendazole in 15.79% (3/19) of the children and 27.19% (31/114) of the adults while mebendazole was received only by 15.79% (3/19) of the children. The association of albendazole with corticotherapy was recommended in 52.63% (10/19) of the children and 56.14% (64/114) of the adults, while mebendazole was associated with corticotherapy in 15.79% (3/19) children and 16.66% (19/114) for adults.

Most patients had a severe clinical form of the disease for 52.63% (10/19) of children and 57.02% (65/114) of adults followed by a moderately severe clinical form in 36.84% (7/19) among children and 32.46% (37/114) among adults.

The majority of the sources of infection were pork in 89.47% (119/133), meat products of wild boar 5.26% (7/133) and a mixture of pork and wild boar products at 0.75% (1/133). In six patients (4.51%), the source of infection was not mentioned in the database or patients' files. A strong association with pork meat products as the source of infection was observed in statistical analyses conducted on patients who met the epidemiological criteria of exposure to a common source of infection (OR=16.29; 95% CI: 4.63–57.32;  $p<0.001$ ).

## **SEROPREVALENCE AND RISK FACTORS OF *TRICHINELLA SPIRALIS* INFECTION IN BLOOD DONORS FROM WESTERN ROMANIA**

This study evaluated the prevalence of anti-*Trichinella* antibodies and the risk factors associated with seroprevalence in blood donors from Western Romania.

The sera of 1347 consecutive blood donors attending the Regional Blood Transfusion Center in Timișoara, Romania were investigated to determine the presence of *T. spiralis*



antibodies. Nova Lisa IgG enzyme immunoassays (ELISA) (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany) were used according to the manufacturer's instructions to detect the presence of anti- *T. spiralis* antibodies in serum samples.

One thousand three hundred forty-seven study participants who were tested for the presence of anti-*Trichinella* antibodies were aged between 18–63 years (mean age = 33.58; SD±10.90) and IgG anti-*T.spiralis* antibodies were detected in 2% of the tested participants. Antibodies against *T. spiralis* were detected in 2.52% of males (19/755) and in 1.35% of females (8/592)( $p=0.13$ ). IgG antibodies were found to be more prevalent in rural blood donors 2.45% (9/368) than in urban blood donors 1.84% (18/979), but it was not linked to *Trichinella* infection ( $p=0.47$ ).

The seroprevalence of *Trichinella* varied depending on the levels of education of study participants as follows:primary education 3.33% (1/30), lower secondary education 1.85% (1/54), vocational school 3.33% (4/120), high school 0.66% (3/453), post-secondary school 3.85% (2/52) and bachelor studies/postgraduate 2.51% (16/638). There was no link between the employment category and *T. spiralis* IgG antibodies based on the data analysis. *T. spiralis* IgG antibodies were not associated with the ownership of a household, in 2.78% (11/395) and pig farming in 1.65% (2/121) respectively (**Table 1**).

**Table 1. Risk factors for *Trichinella* infection in blood donors ascertained by questionnaire.**

Potential Risk Factor	Variables	<i>Trichinella</i> seropositive (n = 27)	<i>Trichinella</i> seronegative (n = 1320)	(95% CI)			p-Value
				OR	Lower Limit	Upper Limit	
Occupation	Owning a household	11	384	1.67	0.77	3.64	0.188
	Pig husbandry	2	119	0.80	0.18	3.45	0.77
Food habits	Eating raw and/or undercooked meat (yes vs. no)	23	546	8.15	2.80	23.70	<0.001
	Eating raw and/or undercooked pork meat	16	387	3.50	1.61	7.62	<0.001
	Eating raw and/or undercooked wild boar meat	21	305	11.64	4.65	29.11	<0.001
	Strong alcoholic drink consumption	7	137	3.02	1.25	7.27	0.009

CI, confidence interval; OR,Odds ratio;

The highest risk factor for acquiring *Trichinella* infection was eating raw and/or undercooked meat 4.04% (23/569;  $p<0.001$ ), from wild boar 6.44% (21/326;  $p<0.001$ ) or pork 3.97% (16/403;  $p<0.001$ )(**Table 1**). *T.spiralis* IgG antibodies seropositivity was highly

associated with strong alcoholic drink consumption 4.86% (7/144)( $p=0.009$ )(**Table 1**). According to *Trichinella* seropositive respondents, they were not diagnosed (81.48%; 22/27) or did not realize that they had *Trichinella* infection (18.51%; 5/27) and did not receive specific therapy (100%; 27/27).

## **ASSESSMENT OF TRICHINELLOSIS KNOWLEDGE AMONG BLOOD DONORS FROM WESTERN ROMANIA**

This study evaluated the knowledge of this disease among blood donors from Western Romania. A questionnaire was conducted among 1347 consecutive blood donors attending the Regional Blood Transfusion Center in Timișoara, Romania between 19 November 2018 and 21 December 2018.

Blood donors were asked to answer to the following seven questions:

1. Have you ever heard of trichinellosis ?
2. Where did you find out about the disease ?
3. How can trichinellosis be contracted ?
4. What are the possible animal sources of *Trichinella* infection ?
5. What are the possible food sources of *Trichinella* infection ?
6. Is there a treatment for trichinellosis in humans ?
7. Can trichinellosis cause death?

To evaluate the level of knowledge of the disease, the questionnaire included questions to which it was possible to answer with "Yes"; "No" or "I don't know". There were also more complex questions with several answer options.

The results of this study suggest that the vast majority of blood donors who answered the questions have heard about trichinellosis and their number significantly increases with age.

Study participants who own a household, with higher education or who are employed seem to be significantly better informed about trichinellosis compared to other categories.

Study participants who are employed may have access a wide range of resources and health information programs at work or within their immediate environment.

The sources of information about this zoonosis seem to be health specialists or the press. Increasing age, rural respondents as well as study participants who were retired were significantly informed about trichinellosis from newspaper; television; physician or veterinarian.

The correct answers regarding how this disease can be contracted significantly increases with age of participants. Participants who are employed and those who have a high

educational level are categories that can be advantaged in terms of access to information with an important role in a better understanding of the transmission of *Trichinella* infection.

The results suggests that with increasing age there is a possible accumulation over time of knowledge about the risks associated with the consumption of insufficiently thermally prepared meat or meat products, thus the study participants becoming more aware and able to prevent *Trichinella* infection.

The results of this study suggest that most study participants who answered to the question know that there is treatment for *Trichinella* infection and their number increases significantly with age. This observation may indicate that as they get older, they may acquire more knowledge about health and available treatments, but they could also have health problems, including parasitic infections, thus increasing the chances of seeking medical assistance and following drug treatment.

Most of study participants who answered to the question, know that trichinellosis can cause death and the results suggest that their number increases significantly with age. This observation could indicate that as they get older, study participants acquire more information from personal experience, medical literature or other informational sources and may be more concerned about diseases and their consequences. Male study participants as well as those who do not own a household, those with a higher level of education and those who are employed, represent the highest proportions of study participants who know that trichinellosis can cause death. Study participants who were employed, those who had a high educational level and those who did not own a household may have superior knowledge and a better understanding of trichinellosis lethal potential.

## **Conclusions and original contributions**

The results of this scientific research bring new and important information regarding the epidemiology of *Trichinella* infection, given that the seropositive subjects have not been previously diagnosed and have not received treatment for this disease thus demonstrating that the infection can be detected in asymptomatic and apparently healthy individuals.

This PhD thesis identified and evaluated the medical records of hospitalized and diagnosed patients with trichinellosis both in children and adults, but also evaluated the main sources of infection, in Western Romania.

In Romania, the level of knowledge regarding trichinellosis has not been previously evaluated. From this perspective, this scientific research presents new and valuable data on the level of trichinellosis knowledge among blood donors in Western Romania, assessed by a questionnaire.

Based on the results presented in this doctoral thesis, the following general conclusions may be highlighted:

1. *T. spiralis* IgG antibodies of the blood donors attending the Regional Blood Transfusion Center in Timișoara, Romania, were detected in 2.00%.
2. The seroprevalence for *Trichinella* tended to increase with age.
3. The prevalence of IgG antibodies was higher in blood donors from rural areas compared to those from urban areas.
4. Eating pork or wild boar was the highest risk factor for contracting *Trichinella* infection.
5. The consumption of strong alcoholic drinks was strongly associated with the presence of *T. spiralis* IgG antibodies.
6. The incidence of trichinellosis infection reached zero in Western Romania at the end of the study period after a trend that has been constantly decreasing, both in children and in adults.
7. The general clinical picture of patients hospitalized and diagnosed with trichinellosis was in accordance with the pattern of symptoms reported in the literature (digestive symptoms, fever and facial edema).
8. Most cases of trichinellosis were represented by severe and moderately severe clinical forms and all required hospitalization.
9. The highest proportions of elevated laboratory findings for cases hospitalized and diagnosed with trichinellosis (number of eosinophils; number of leukocytes; erythrocyte sedimentation rate) were among adults.
10. Arad was the county with the highest incidence (number of cases per 100,000 inhabitants) of trichinellosis cases for the study period.
11. The most common source of infection was pork, followed by wild boar meat and the combination of both meat products.

12. Most hospitalized cases were residents of rural areas and a significant number of patients were part of various family outbreaks.

13. The results of the study show that winter was the season with the highest number of trichinellosis cases.

14. Given the severity of complications that may occur during the disease's evolution, consuming tested meat in accordance with the Commission Regulation 2015/1375 rule and testing by ISO 18743:2015 is highly recommended.

15. Most donors who answered the questionnaire were well-informed about the animal, food sources for *Trichinella* infection, how this disease is transmitted, the existence of drug treatment as well as the lethal potential of this disease.

16. A higher level of education, having a job or a household significantly increased the chances of being well informed about *Trichinella* infection.

17. The written or televised media, as well as health specialists, are the most frequently used sources of information.

18. A significant percentage of donors do not know that there is a treatment for trichinellosis or that this zoonosis can cause death.

19. Education of the population adapted to its level of understanding, regarding their food habits, in particular undercooked or incorrectly processed meat, or raw meat consumption, play a key role to fully prevent trichinellosis in Western Romania.

20. To completely prevent the disease and to increase the level of knowledge about *Trichinella* infection, information must be accessible and effective ways of communication must be maintained.

It is necessary to increase the awareness of *Trichinella* infection among people with a lower socio-economic level, who are unemployed, and with a low level of education, but also among young people, especially since this last category can have native digital skills and easier access to information.

The results of the studies carried out in this doctoral thesis provide an overview of trichinellosis in terms of the seroprevalence and level of knowledge of the disease among blood donors, but also the incidence of the disease in Western Romania, which can contribute to a better implementation of the present methodology for surveillance and control of

trichinellosis in humans, but also for the development of an accessible information and prevention guide.