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### Original Research Article

# Seroprevalence and Risk Factors of *Trichinella spiralis* infection among Pregnant Women in Rural Areas, Burkina Faso

Marc Christian Tahita<sup>1,2\*</sup>, Ousmane Traore<sup>1,2</sup>, Bérenger Kabore<sup>1,2</sup>, Hamidou Ilboudo<sup>1,2</sup>, Regina Sinner<sup>3</sup>, Adama Kazienga<sup>1,2</sup>, Hyacinthe Sanou<sup>1,2</sup>, Palpouguini Lompo<sup>1,2</sup>, Judith M. Hübschen<sup>3</sup>, Halidou Tinto<sup>1,2</sup>

<sup>1</sup>Institut de Recherche en Sciences de la Santé/Direction Régionale du Centre-Ouest, Nanoro, Burkina Faso

<sup>2</sup>Clinical Research Unit of Nanoro, Nanoro, Burkina Faso

<sup>3</sup>Clinical and Applied Virology, Department of Infection and Immunity, Luxembourg Institute of Health

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Abstract: Trichinellosis is a serious zoonotic disease with worldwide distribution caused by parasitic nematodes of the genus Trichinella. If not well managed, this can lead to complications and death. Data in Burkina Faso are scarce. Therefore, this study aimed to assess the seroprevalence and risk factors of Trichinella infection in Burkina Faso. This was a cross-sectional study in which pregnant women attending antenatal care were enrolled after signature of an informed consent. Serum samples of 418 pregnant women were collected and stored at the Clinical Research Unit of Nanoro. Anti-Trichinella spiralis IgG Human ELISA Kit was used to test the samples at the Luxembourg Institute of Health. Anthropometric, clinical and socio-demographic data were collected using a structured questionnaire and Chi-square and Student t-tests were used to compare groups. The p-value significance threshold was set at 0.05. Overall, the seroprevalence of trichinellosis was 2.4% (10/418). The baseline characteristics analysis showed associations between Trichinella seropositive pregnant women and factors such as body mass index (BMI) and gravidity. Individuals identified as *Trichinella* seropositive were not previously diagnosed and have not been treated for Trichinella infection with any specific therapy. A low seroprevalence of *Trichinella* infection was found in our study area. Efforts for continuous education in trichinellosis prevention are

**Keywords:** *Trichinella Spiralis*, Prevalence, Risk factors, Pregnant women, Nanoro, Burkina Faso.

Running title: *Trichinella* Seroprevalence in Pregnant Women.

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#### Introduction

About 10,000 cases of trichinellosis occur worldwide each year (Capó & Despommier, 1996; Gottstein et al., 2009). Trichinellosis is a disease caused by the larvae, 'trichinae', of a small nematode worm (*Trichinella spiralis*), which can affect many species including humans and animals (Huong et al., 2017). Human trichinellosis is caused by consuming raw or inadequately treated meat from domestic or game animals containing the larvae of parasites of the *Trichinella* species (CDC, 2024). Most of the eight species are widely distributed geographically, but few are found only in specific areas and animals (Pozio & Zarlenga, 2005). Once infected, about 50% of patients present with conjunctivitis and subconjunctival hemorrhages (Bruschi & Murrell, 2002). Splinter

hemorrhages on nailbeds (subungual) and retinal hemorrhages can also occur (Capó & Despommier, 1996; Gottstein et al., 2009; Huong et al., 2017). In most cases, the disease is self-limited but systemic complications are treated with antiparasitic agents and corticosteroids (Watt et al., 2000). The main morbidities due to Trichinella spiralis are myocarditis, pneumonitis, secondary bacterial pneumonia, nephritis, chronic diarrhea and neurotrichinellosis (Rawla & Sharma, n.d.). Despite the availability of some treatments, mortality occurs in 5% of severe cases (Lupu et al., 2021). Trichinellosis is thus a public health issue given its health and economic impact in low- and middle-income countries (LMICs). Data on the epidemiology and burden of infection in humans are scarce and underreporting is common due to the lack of knowledge of this parasitic disease for clinical diagnosis (Pavel et al., 2022). Moreover, the lack of adequate serological tests combined with high costs do not favor routine diagnosis of this disease. Trichinellosis control in humans relies on three main approaches: education about the consumption risk of raw or semi-raw meat, farming of pigs under strict veterinary control and control of all susceptible animals by a standardized artificial digestion method at slaughtering or after hunting (Gottstein et al., 2009). In Burkina Faso, studies on the prevalence of this foodborne disease are scarce and when present, focus on animals. The prevalence and risk factors of Trichinella infection in pregnant women, a risk group, are unknown. Therefore, the present study was conducted to evaluate for the first time the seroprevalence and risk factors of Trichinella infection in pregnant women attending antenatal care for their first time in Burkina Faso.

# MATERIALS AND METHODS

#### Study Design, Area, and Population

The Clinical Research Unit of Nanoro conducted this study in the catchment area of the Nanoro Health District. The inhabitants are mostly composed by subsistence farmers and cattle-keepers (50.6%), housewives (31.2%), scholars (9.6%) and civil servants (2.5%) (Derra *et al.*, 2012). The poor quality of drinking water and individual and collective hygiene, associated with poverty, are the main reasons for health problems in the area. The general morbidity is high and malaria accounts for about 40% of the total burden of disease.

#### **Sample Size Calculation**

This is an ancillary study of a main study aiming to improve the case detection and management of malaria cases early in pregnancy. There is no data on the prevalence of *Trichinella spiralis* in Burkina Faso. Using a single population proportion formula with 43% prevalence, 95% confidence level, 5% margin of error, and 10% non-response rate the estimated total sample size was 418.

#### **Data collection**

Briefly, all pregnant women attending antennal care (ANC) for their first time were asked to participate in the study by the clinical team. After obtaining written informed consent, a physical examination was conducted. A structured questionnaire was used to capture sociodemographic information, the level of knowledge about trichinellosis and the risk factors.

#### Sample collection and laboratory methods

Venous blood was collected in serum separator tubes (SST<sup>TM</sup> II advance, BD Vacutainer® SST) and thereafter transferred to the Clinical Laboratory of the CRUN for sera collection and storage at -80°C. The

samples were shipped to the Luxembourg Institute of Health where ELISA was performed. The serum samples were tested using Anti-Trichinella spiralis IgG Human ELISA Kit (Abcam 108780, United Kingdom) for the presence of immunoglobulin G (IgG) antibodies against T. spiralis (Specificity 94.8%, Sensitivity >95%), according to the manufacturer's instructions. The controls provided in the kits were used in each analysis. They included a Trichinella spiralis IgG positive control, IgG Cut-off control and an IgG negative control. The samples were considered positive if the absorbance value was more than 10% over the cut-off value. Samples with an absorbance value of less than 10% above or below the cut-off control value were considered inconclusive (grey zone). Those samples were retested, and the second test result was considered the final ELISA result. Samples were considered negative if the absorbance value was more than 10% below the cut-off.

### **Statistical Analysis**

Data were double-entered using Open Clinica, cleaned and analyzed with Stata 12 software (Stata Corp., College Station, TX, USA). Chi-squared and Student t-tests were used for intergroup comparisons. The significance level of the statistical tests was 5%.

# RESULTS

# Socio-demographic and clinical characteristics of the study population

Overall, 418 pregnant women were enrolled in the study. The mean age of participants was 26 years with a standard deviation of 6.6 years. Age distribution showed that 10.5% of the overall population was between 16 and 18 years old and represented up to 40.4% of the primigravidae. More than three-quarters of the pregnant women (75.6%) were aged between 18 and 34 years, with respectively 59.6% of primigravidae, 98.2% of secundigravidae, and 77.5% of multigravidae falling into this age range. Only 13.9% were between 34 and 45 years old including 22.5% of multigravidae with no primigravidae. Educational levels varied significantly, with 69.9% having no education, 9.1% attaining primary level, and 21.1% having secondary education or higher. The majority of multigravidae (87.7%) had no education, while a higher percentage of primigravidae (47.7%) had secondary education or higher. Regarding occupation, most of the women were housewives (78.0%), with this being the most common occupation across all gravidity groups. Farmers made up 6.0%, pupils 5.7%, and informal sector/civil servants 10.3%. The mean body mass index (BMI) was 21.9 (SD: 2.6). and varied across the gravidity groups. Almost 14% of the pregnant women were in their first trimester, 79.4% in their second trimester, and 6.6% in their third trimester.

Table 1: Socio-demographic and clinical characteristics of pregnant women at enrolment according to gravidity

	Overall	Primigravidae	Secundigravidae	Multigravidae
n	418	109	56	253
Age (mean (SD)	26.00 (6.59)	19.06 (1.74)	22.61 (3.61)	29.73 (5.50)
Age group (%)				
[16,18]	44 (10.5)	44 (40.4)	0 (0.0)	0 (0.0)
(18,34]	316 (75.6)	65 (59.6)	55 (98.2)	196 (77.5)
(34,45]	58 (13.9)	0 (0.0)	1 (1.8)	57 (22.5)
Educational level (%)				
None	292 (69.9)	42 (38.5)	28 (50.0)	222 (87.7)
Primary	38 (9.1)	15 (13.8)	5 (8.9)	18 (7.1)
Secondary and tertiary	88 (21.1)	52 (47.7)	23 (41.1)	13 (5.1)
Occupation (%)				
Farmer	25 (6.0)	4 (3.7)	0 (0.0)	21 (8.3)
Pupil	24 (5.7)	19 (17.4)	4 (7.1)	1 (0.4)
Housewife	326 (78.0)	74 (67.9)	45 (80.4)	207 (81.8)
Informal Sector/Civil Servant	43 (10.3)	12 (11.0)	7 (12.5)	24 (9.5)
BMI (mean (SD)	21.95 (2.57)	21.63 (2.49)	21.84 (2.20)	22.12 (2.67)
Trimester (%)				
1 <sup>st</sup> trimester	58 (13.9)	19 (17.4)	11 (19.6)	28 (11.1)
2 <sup>nd</sup> trimester	332 (79.4)	86 (78.9)	44 (78.6)	202 (79.8)
3 <sup>rd</sup> trimester	28 (6.7)	4 (3.7)	1 (1.8)	23 (9.1)

#### Seroprevalence of Trichinella spiralis

The overall seroprevalence of *Trichinella* spiralis in the study was 2.4%, with a 95% confidence interval of 1.2 to 4.4%. The study included 418 pregnant women, with 10 testing positive for Trichinella antibodies (Table 2). Most positive cases (80%) occurred in women aged 18 to 34 who were also in their second trimester of pregnancy; however, statistical analysis revealed no significant difference in seroprevalence

between age groups (p = 0.932) or pregnancy trimesters (p = 0.870). In contrast, there was a significant association between parity and BMI. Women in their second (secundigravidae) or subsequent (multigravidae) pregnancies had a significantly higher proportion of positive cases than those in their first pregnancy (primigravidae). Furthermore, the BMI category showed significant differences in infection rates (p = 0.028).

Table 2: Seroprevalence of *Trichinella* among pregnant women attending antenatal care for the first time

	Overall	Seroprevalence		p
		Negative	Positive	
n	418	408	10	
Age group (%)				0.932
[16,18]	44 (10.5)	43 (10.5)	1 (10.0)	
(18,34]	316 (75.6)	308 (75.5)	8 (80.0)	
(34,45]	58 (13.9)	57 (14.0)	1 (10.0)	
BMI group (%)				0.028
(0, 18.5]	25 (6.1)	25 (6.3)	0 (0.0)	
(18.5, 24.5]	333 (81.4)	324 (81.2)	9 (90.0)	
(24.5,29.5]	47 (11.5)	47 (11.8)	0 (0.0)	
(29.5, 35]	4 (1.0)	3 (0.8)	1 (10.0)	
Trimester (%)				0.870
1 <sup>st</sup> trimester	58 (13.9)	57 (14.0)	1 (10.0)	
2 <sup>nd</sup> trimester	332 (79.4)	324 (79.4)	8 (80.0)	
3 <sup>rd</sup> trimester	28 (6.7)	27 (6.6)	1 (10.0)	
Gestity (%)				0.037
Primigravidae	109 (26.1)	108 (26.5)	1 (10.0)	
Secundigravidae	56 (13.4)	52 (12.7)	4 (40.0)	
Multigravidae	253 (60.5)	248 (60.8)	5 (50.0)	

# Monthly variation of *Trichinella* seroprevalence according to recruitment period

During the period spanning from November 2020 to March 2021, the monthly variation of *Trichinella spiralis* exposure was monitored and is reflected in the provided data. An increase was observed in exposure of *Trichinella* in December 2020 representing 0.8%

(1/133). This pattern was sustained into January 2021, where 3.0% (4/135) of pregnant women were seropositive. The trend remained unchanged in February 2021 showing 3.3% of seropositive cases of *Trichinella*. The month of March 2021 marked a notable decline with only 1 case of exposure representing 4.3% (1/23) of the pregnant women seen during this month (Figure 1).

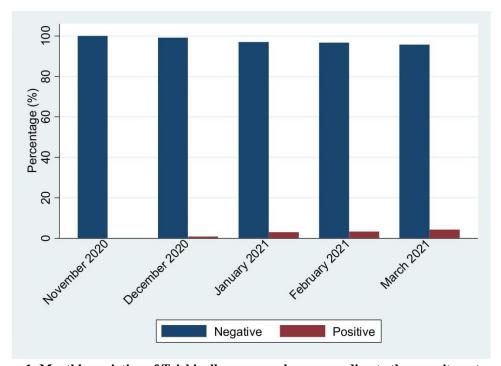


Figure 1: Monthly variation of Trichinella seroprevalence according to the recruitment period

# **DISCUSSION**

This present study was conducted to evaluate for the first time, the seroprevalence and associated factors of Trichinella spiralis infection in pregnant women attending their first antenatal care. Although this study was limited to a rural area and pregnant women, the low prevalence of 2.4% suggests a low transmission in the study area. To date, this study represents the first assessment of human seroprevalence of Trichinella spiralis in Burkina Faso. On one hand, this low prevalence of Trichinella can be attributed to the less common home slaughter without veterinary supervision and on the other hand to generally improved conditions in water sanitation and hygiene (WASH). The global seroprevalence of Trichinella spiralis considerably across regions. In neighboring Ghana, a low seroprevalence of 0.34% was observed in humans (Addo et al., 2021). Similarly, other studies in Ethiopia reported low seroprevalence ranging from 0.02% to 0.04% (Murrell et al., 2011). Latin American countries, including Mexico (1.0% to 1.9% with De-La-Rosa et al., (de-la-Rosa et al., 1998)) and Chile (1.5% with Contreras et al., (Contreras et al., 1994), also reported low seroprevalence. In contrast, Northern Laos exhibited a

high seroprevalence of 19.1% (Conlan et al., 2014). Accurately determining the prevalence of trichinellosis in humans is challenging due to the unspecific nature of the disease, which clinically resembles to typhoid fever, leading to potential misdiagnosis, particularly in resource-poor settings (Addo et al., 2021). One of the primary causes of Trichinella infection is reported to be the consumption of raw and/or undercooked meat from pigs or wild boars (Antolová et al., 2018; Cui et al., 2011). Thus, prevention and control of trichinellosis rely on education and hygiene in the environment and at home (Foreyt et al., 2013). It has been demonstrated that heating meat to at least 70°C in the center will effectively kill Trichinella larvae (Gamble et al., 2000). In the context of the present study, the observed low seroprevalence can be attributed, firstly, to the low consumption of meat in rural areas and, secondly, to culinary habits in Burkina Faso where the consumption of raw or undercooked pork is uncommon. Additionally, religious beliefs, with one-third of the study population abstaining from pork consumption, contribute to this relatively low seroprevalence.

Our results also revealed that *Trichinella* seroprevalence tended to increase with age with the age

group 19-45 having the highest seroprevalence. This range of population is the one who is more likely to eat meat. Since IgG antibodies were detected, a higher age would mean a higher risk of exposure sometime during the life (lifetime exposure). This was also confirmed in the study by Cui *et al.*, (Cui *et al.*, 2011). Therefore, public health strategies based on education to eliminate the risk of exposure in this age group would be worth. Trichinellosis complications such as myocarditis or encephalitis are generally fatal in elderly individuals (Ruetsch *et al.*, 2016) and can worsen health conditions in pregnancy as well.

The analysis of baseline characteristics also revealed statistically significant differences for body mass index (BMI) and gravidity among *Trichinella* seropositive women. Higher BMI values (18.5, 24.5) and having been pregnant before showed a potential association with *Trichinella* seropositivity. Unfortunately, no data are available on WASH and nutrition habits since the main study objective was malaria.

Moreover, a higher prevalence was noted among women without formal education. Indeed, if people are not aware that this parasite exists and how to prevent infection, the risk of exposure is higher. Although these observations did not reach statistical significance, they offer valuable insights for subsequent investigations and public health interventions. The observed patterns in age, education, marital status, occupation, and religion suggest that further explorations of these factors to understand their contributions to Trichinella infection risk are needed. Numerous factors, like the family's handling of raw meat and low socioeconomic position, which is typically associated with subpar WASH conditions, can be used to explain this. Furthermore, people who avoid eating domestic pigs have a lower risk of contracting trichinellosis than people who do. In the present study, a low seroprevalence of Trichinella spiralis was found in Burkina Faso. This will inform targeted interventions, raising awareness and aiding in the prevention and control of Trichinella infections. However, the finding that healthy, asymptomatic people had evidence of previous infection with this zoonosis suggests that further prevalence studies are required.

#### **Ethics Approval and Informed Consent**

The present study was approved by the Comité d'Ethique Institutionnel pour la Recherche en Santé (N/Réf. A15-2020/CEIRES) and the study was conducted under good clinical practices. All participants were duly informed of the study's objectives and the protocol for sample collection. Written informed consent was obtained from each study participant. Participation was voluntary.

#### **Authors' Contributions**

TMC and TH conceptualized the study. TMC, TH, TO, and KB designed the study. IH, LP and SH participated in fieldwork and data collection. HJM and SR performed the laboratory analysis. KA performed the data analysis; TMC, TH, TO, KB, HJM and SR interpreted the data. TMC prepared the first draft of the manuscript, reviewed by TH, TO, KB, IH, HJM, SR, LP, SH.. All authors contributed to the development of the final manuscript and approved its submission. All authors have read and agreed to the published version of the manuscript.

#### Disclosure of conflict of interest: None

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