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ABSENCE OF *Trichinella* spp. ANTIBODIES IN NORTHERN PERUVIAN AMAZON PECCARIES

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ABSTRACT

Trichinellosis is a global parasitic food-borne disease affecting humans, domestic and wild animals. Beyond its health implications, trichinellosis also has negative economic impacts for the pork industry. However, to date, few studies have analyzed *Trichinella* infections in the Amazon region. This study aimed to increase the information on the circulation of *Trichinella* spp. in free-ranging peccaries in two areas in the Northern Peruvian Amazon. Between 2008 and 2020, local subsistence hunters collected dried blood spots on filter paper from 140 free-ranging *Pecari tajacu* and 93 *Tayassu pecari* (n= 233 total), to test for

antibodies against *Trichinella* spp. All animals were negative in serological tests for anti-*Trichinella* spp. antibodies. No evidence of nematodes was also found in the histological sections of the analyzed peccary diaphragm samples (n=29). In the two study areas, free-ranging peccary populations do not show evidence of having been exposed to the parasite. This is the first survey of *Trichinella* spp. in wild peccaries conducted in the Amazon. Negative-result studies offer key insights into trichinellosis distribution.

KEYWORDS: Trichinellosis, food-borne disease, zoonosis, Tayassuidae, Amazon.

AUSENCIA DE ANTICUERPOS CONTRA *Trichinella* spp. EN PECARIES DE LA AMAZONIA NORTE PERUANA

RESUMEN

La triquinosis es una enfermedad parasitaria global transmitida por los alimentos que afecta a los seres humanos, animales domésticos y salvajes. Más allá de sus implicaciones para la salud, la triquinosis también tiene impactos económicos negativos para la industria porcina. Sin embargo, hasta la fecha, pocos estudios han analizado las infecciones por *Trichinella* en la Amazonía. Este estudio tuvo como objetivo aportar nueva la información sobre la circulación de *Trichinella* spp. en pecaríes de vida libre en dos áreas del norte de la Amazonia peruana. Entre 2008 y 2020, cazadores de subsistencia locales recolectaron muestras de sangre seca en papel de filtro de 140 *Pecari tajacu* y 93 *Tayassu pecari* (233 en total) en libertad para analizar anticuerpos contra *Trichinella* spp. Todos los animales resultaron negativos en las pruebas serológicas de anticuerpos contra *Trichinella* spp. Tampoco se encontró evidencia de nematodos en las secciones histológicas de las muestras de diafragma de pecarí analizadas (n=29). En ninguna de las dos áreas de estudio, las poblaciones de pecaríes de vida libre mostraron evidencias de haber estado expuestas al parásito. Este es el primer estudio sobre *Trichinella* spp. en pecaríes de vida libre realizado en la Amazonía. Los estudios con resultados negativos ofrecen información clave sobre la distribución de la triquinosis.

PALABRAS CLAVE: Triquinosis, enfermedad alimentaria, zoonosis, Tayassuidae, Amazonía.

INTRODUCTION

Trichinellosis is a neglected and emerging food-borne parasitic disease of significant global public health concern (Bruschi, 2012). Human trichinellosis is estimated to affect 2,700–10,000 people annually worldwide, with a mortality rate ranging from 0.06% to 0.2% (Pozio, 2021). However, the true burden and the disease's occurrence are likely underestimated due to the lack of reliable diagnostic tools in some areas in remote regions (Pozio, 2021), such as the Amazon region.

Humans become infected with *Trichinella* by eating raw or undercooked meat containing encysted larvae, most often from pork or wild game—such as foxes, cougars, badgers, deer, goats, and armadillos—, an exposure frequently linked to traditional or cultural food practices (CDC, 2024; Pozio, 2021). The trichinellosis symptoms vary depending on the stage of infection. Early signs may include mild, transient diarrhea and nausea, while more severe cases can lead to myocarditis, which has been associated with fatalities (Kocięcka, 2000). Beyond its health implications, trichinellosis also has negative economic impacts for the pork industry (Ribicich *et al.*, 2020).

Trichinella parasites have been reported in both domestic and wild animals across all continents, circulating among carnivorous and omnivorous vertebrates in both domestic and sylvatic environments (Dupouy-Camet & Murrell, 2007; Pozio, 2000). However, in South American countries, limited data underscores the need for further research to fully understand the circulation of *Trichinella* spp. in the region (Ribicich *et al.*, 2020).

Trichinellosis is a public health concern, particularly in Argentina and Chile, where it has been frequently reported in humans (Ribicich *et al.*, 2020), and in domestic pigs and wild animals—such as armadillos, peccaries, wild boars, and

carnivores— (Hidalgo *et al.*, 2019; Krivokapich *et al.*, 2006; Landaeta-Aqueveque *et al.*, 2015; Reissig *et al.*, 2024). Pigs and wild boars have been reported to carry the highest parasitic loads of *Trichinella* spp., while armadillos and peccaries exhibit lower larval burdens (Ribicich *et al.*, 2020). In Bolivia, Brazil, and Ecuador, trichinellosis appears to be less common. However, *Trichinella* antibodies have been detected in various hosts, including pigs in Bolivia (Macchioni *et al.*, 2012) and Ecuador (Chávez-Larrea *et al.*, 2005), and wild boars in Brazil (Gomes, 2023; Silva *et al.*, 2022).

In the Amazon, wild meat represents a significant source of animal protein and income for rural and urban people, and peccaries (Suborder Suinae, Family Tayassuidae) are among the most hunted and consumed species (El Bizri *et al.*, 2020; Mayor *et al.*, 2022). Thus, their frequent consumption poses a significant risk factor for parasitic infections. Although peccaries have been reported as natural hosts of *Trichinella* spp. in other South American countries, no studies have been conducted on its circulation in the Amazon region, probably hampered by logistic limitations of studying wildlife diseases in remote environments (Menajovsky *et al.*, 2023). This study aimed to provide information on the circulation of *Trichinella* spp. in free-ranging populations of peccaries hunted for self-consumption in two areas in the Northern Peruvian Amazon.

MATERIALS Y METHODS

STUDY AREA

The study was conducted in the community of Nueva Esperanza and the Pucacuro National Reserve. Nueva Esperanza is a Yagua indigenous community of approximately 300 people, situated in the Yavarí-Mirín River basin (04°19'53"S; 71°57'33"W), a remote and well-preserved

upland forest along the Peru-Brazil border. The primary activities of this community include traditional small-scale agriculture, fishing, logging, and subsistence hunting (Mayor *et al.*, 2015). The Pucacuro National Reserve (2°26'53"S 75°20'29"W), a protected area characterized by its humid tropical forest landscape, implements a game species management program, allowing indigenous groups to hunt certain species for both subsistence and commercial purposes (SERNANP, 2014; Figure 1).

The climate in the region is typically equatorial with annual temperatures ranging from 22°C–36°C, a relative humidity of 80%–100%, and annual rainfall of 1,500–3,000 mm.

BIOLOGICAL COLLECTION

Between 2008 and 2020, local subsistence hunters in both areas collected blood samples from the cranial or caudal vena cava onto Whatman filter paper No.3, Whatman 903 Protein Saver cards or FTA® cards (Scheilcher & Schuell, Dassel, Germany) from 233 peccaries, including collared peccaries (*Pecari tajacu*, n=140) and white-lipped peccaries (*Tayassu pecari*, n=93), as part of a wildlife conservation program. In average, the samples were stored at room temperature for 123 ± 101 days in the study area before being transferred and preserved at -70°C for later analysis. In parallel, diaphragm samples from 19 *P. tajacu* and 10 *T. pecari* were also collected and

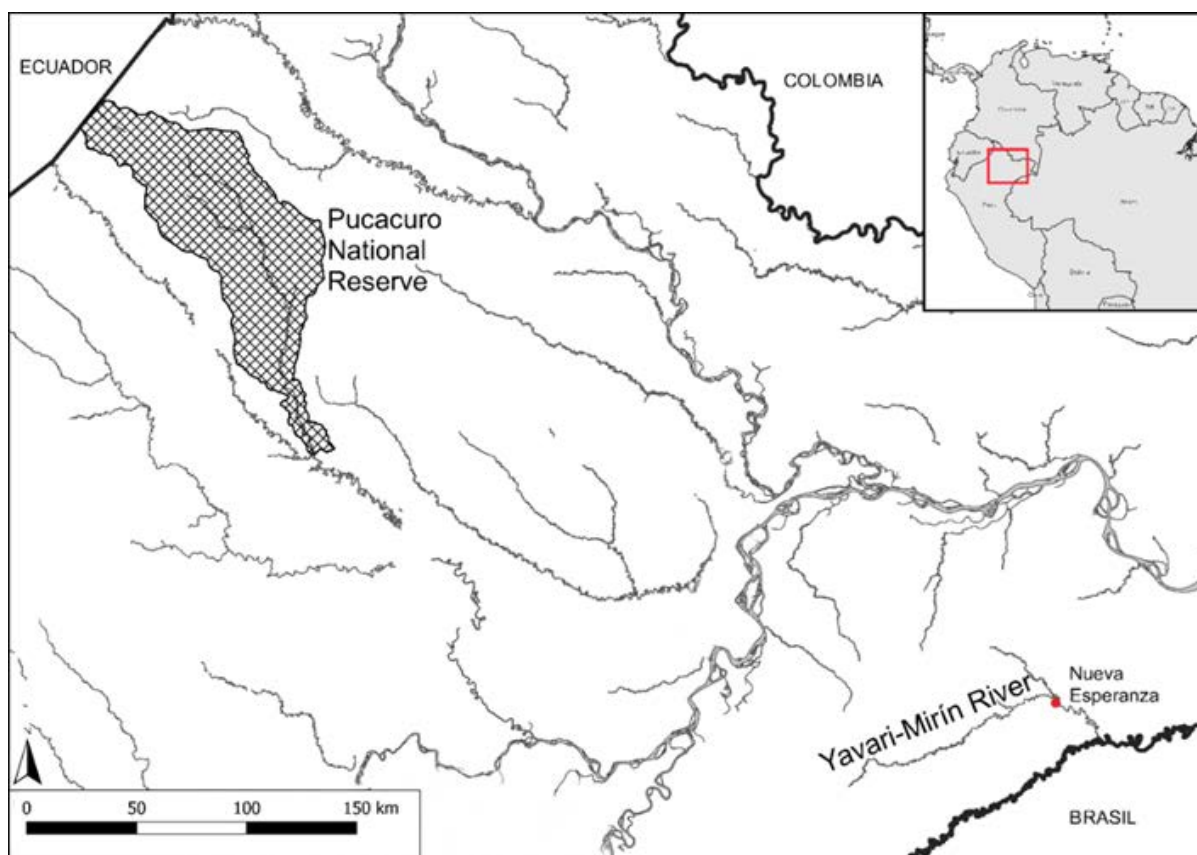


Figure 1. Map of the study area, showing the location of the community of Nueva Esperanza in the Yavari Mirín River basin and the Pucacuro National Reserve in the Northern Peruvian Amazon.

preserved in buffered 4% formaldehyde solution (v/v).

LABORATORY ANALYSES

A 132 mm² section of blood-soaked filter paper was cut and diluted in 400 µL of sterile phosphate-buffered saline (PBS). The samples were vortexed for 20 seconds, stored at 4°C for 24 hours, vortexed again, and then frozen at -20°C for later analysis. The elutions were tested for antibodies against *T. spiralis*, *T. pseudospiralis*, *T. britovi*, and *T. nativa*, using the ID Screen® *Trichinella* Indirect Multi-species kit (IDvet, Montpellier, France), following established protocols (Menajovsky *et al.*, 2024).

A histopathological analysis of diaphragm samples was performed. The samples were stained with hematoxylin and eosin, and subsequently observed under a microscope (Leica DM500) at magnifications of 10x and 40x.

RESULTS AND DISCUSSION

Our study shows that free-ranging peccaries studied for 12 years in two areas in the Northern

Peruvian Amazon have not been exposed to the parasite, as no antibodies were detected. Also, no evidence of nematodes was found in the histological sections of the analyzed peccary diaphragm samples (Table 1).

Trichinellosis is a globally significant food-borne parasitic disease transmitted primarily through the consumption of raw or improperly processed meat, yet it remains understudied in South America (Bruschi, 2012; Pozio, 2018; Ribicich *et al.*, 2020). To our knowledge, despite the presence and consumption of domestic pigs and peccaries —both potential hosts of *Trichinella* spp.— no studies have been conducted in the Amazon. Our research addresses this gap, representing the first investigation of *Trichinella* infections in wildlife in the region.

In South America, trichinellosis has been reported mainly in Argentina and Chile, where *Trichinella* spp. has been detected in several animal species, including pigs, dogs, rats, and wild animals such as peccaries, wild boars, armadillos, and carnivores (Echeverry *et al.*, 2021; Hidalgo *et al.*, 2019; Krivokapich *et al.*, 2008, 2015, 2019; 2006; Reissig *et al.*, 2024; Ribicich *et al.*, 2010; 2020). In both countries, the widespread

Table 1: Antibody detection results for *Trichinella* spp. in 140 free-ranging *Pecari tajacu* and 93 *Tayassu pecari* collected in the Yavarí-Mirín River basin and the Pucacuro National Reserve in the Northern Peruvian Amazon.

Area	Species	<i>Trichinella</i> spp.
Yavarí-Mirín River basin	<i>Pecari tajacu</i>	0/137 (0%) [CI _{95%} : 0.00-2.73]
	<i>Tayassu pecari</i>	0/57 (0%) [CI _{95%} : 0.00-6.31]
	Total	0/194 (0%) [CI _{95%} : 0.00- 1.94]
Pucacuro National Reserve	<i>Pecari tajacu</i>	0/3 (0%) [CI _{95%} : 0.00-56.15]
	<i>Tayassu pecari</i>	0/36 (0%) [CI _{95%} : 0.00-9.64]
	Total	0/39 (0%) [CI _{95%} : 0.00-8.97]

circulation of the parasite in these animal populations has led to human infections, with 258 confirmed cases reported in Chile (2005–2015) and 6,662 suspected cases in Argentina between 2012 and 2018 (Ribicich *et al.*, 2020). In Peru, we found only one study investigating *Trichinella* spp. in 185 backyard pigs in Lima using both ELISA and trichinostomy, but found no evidence of infection (Arrese *et al.*, 2014).

The study has a potential limitation due to the prolonged sample preservation time at room temperature, as this could decrease sample quality. Previous studies using the same experimental samples have shown positive ELISA serology results for Aujeszky's disease virus, Classical swine fever virus, Hepatitis B virus, and *Toxoplasma gondii*, even showing high frequencies of antibody presence against *Toxoplasma gondii* (Menajovsky *et al.*, 2024a, 2024b, 2025), suggesting that the negative results obtained in the present study are true negatives rather than a consequence of sample deterioration.

Although the kit has not yet been validated in peccaries, according to the manufacturer, the ELISA kit is specifically designed for use in pigs and wild boars, with its effectiveness has already been demonstrated in numerous pig studies (Eslahi *et al.*, 2022), wild boar populations in Brazil (Silva *et al.*, 2022), and various other wild species across Europe, including raccoons and foxes, which are phylogenetically distant from pigs and wild boars yet still yield reliable results (Cybulska *et al.*, 2016; 2021). However, we cannot rule out false negatives due to the specificity of the kit.

Finally, while serological methods are useful for detecting exposure and have been validated in numerous studies as valuable tools for monitoring and surveillance (Chávez-Larrea *et al.*, 2005; Macchioni *et al.*, 2012; Silva *et al.*, 2022), they are less reliable for detecting low-level infections compared to the gold standard diagnostic method

of artificial digestion, which directly identifies larvae in tissue (WOAH, 2023). Consistently, the negative results of 29 individuals in both serological and in histopathological tests suggest that the negative results are truly negative.

Scientific publications generally focus on highlighting the presence of different pathogens, but herein we claim the importance of communicating the absence of pathogens, as this also helps improve understanding of the geographical distribution of certain diseases. Notably, studies reporting negative results—such as those on pigs, carnivores, and horses in Brazil—(Daguer *et al.*, 2006; de Oliveira Andrade *et al.*, 2025; de Oliveira Souza *et al.*, 2013; Fernandes Neto & Salotti-Souza, 2017; Zolla, 2024), wild boars in Uruguay (Altuna *et al.*, 2018), and pigs and rodents in Colombia (Chaparro-Gutierrez *et al.*, 2018; Laverde *et al.*, 2009), along with the present study—provide valuable insights into the distribution of trichinellosis.

CONCLUSION

Our findings show no evidence that free-ranging peccaries in the Northern Peruvian Amazon have been exposed to *Trichinella* spp. This is particularly relevant given that trichinellosis is a notifiable disease with serious implications for both animal and public health, potentially causing severe illness and even death in humans. Understanding the distribution of this parasite is crucial for enhancing disease control strategies, not only in wildlife but also in human populations and the pork industry, where its presence poses significant risks.

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ETHICS STATEMENT

Field and laboratory procedures were authorized by Dirección General de Flora y Fauna Silvestre from Peru (041-2007- DGGFS-DGEFFS, 0350-2012-DGFFS-DGEFFS, 258-2019-MINAGRI-SERFOR-DGGSPFFS), the Head of the National Reserve of Pucacuro (03-2012-SERNANPRN Pucacuro) and the Institutional Animal Use Ethics Committees from the Universidad Peruana Cayetano Heredia (029-03-19, protocol #102142) and the Universitat Autònoma de Barcelona (protocol #4829). Export permits: 003258-CITES-Perú, 003260-CITES-Perú, BB-00017 20I-Spain and BB-00018 20I-Spain.

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