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Prevalence and Molecular Characterization of Taenia spp. and Seroprevalence of Toxoplasma gondii in Slaughtered Pigs in Burundi

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ABSTRACT

Taenia species and *Toxoplasma gondii* are zoonotic parasites transmitted through food, posing health risks to humans and pigs. In Burundi, data on their prevalence in pigs is scarce. This study aimed to quantify the occurrence of *Taenia* spp. using meat inspection, partial carcass dissection, and molecular methods, and to assess *T. gondii* exposure through serological testing. A cross-sectional survey was carried out in slaughter facilities in Bujumbura city, Kayanza, and Ngozi provinces, sampling 576 pigs through a multisite collection strategy. Blood samples were obtained at slaughter for indirect ELISA targeting the *T. gondii* P30 protein. Routine meat inspection was conducted to identify *T. solium* and *T. hydatigena* cysticerci, while the tongue, heart, and masseter muscles were sliced (<5 mm) to evaluate cysticerci burden and developmental stages. Selected cysticerci and suspicious lesions were analyzed using PCR-RFLP to confirm species identity. Meat inspection detected *T. solium* in 2.4% of pigs, whereas partial carcass dissection revealed 11.6% positive cases. PCR-RFLP confirmed 11.5% as *T. solium*. The average cysticerci count per infected pig was 80, predominantly in the masseter muscles (76.1%), followed by the tongue (18.8%) and heart (5.1%). Most cysticerci (88.3%) were viable, with smaller proportions being calcified (6.4%) or degenerated (5.3%). Infection intensity was light in 69%, moderate in 13.4%, and heavy in 17.9% of infected pigs. *T. hydatigena* was suspected in 5.5% of pigs and confirmed in 4.2%. The seroprevalence of *T. gondii* was 17.7%. Both *T. solium* and *T. gondii* are present in Burundian pigs, highlighting a significant public health concern. Comprehensive control strategies—including improved pig management, hygiene, meat inspection, safe food handling, and treatment of tapeworm carriers—are essential to reduce transmission to humans and livestock.

Keywords: Taenia solium, Taenia hydatigena, Toxoplasma gondii, pigs, Burundi, Zoonosis

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Background

A number of foodborne parasites, notably *Taenia* spp. and *Toxoplasma gondii*, are responsible for considerable public health and economic impacts worldwide [1]. Among these, *Taenia solium* is of particular concern. This tapeworm relies on two different hosts to complete its development: humans harbor the adult stage, whereas pigs—and, in rare situations, humans—carry the larval stage [2]. The parasite persists mainly in resource-limited regions such as large parts of sub-Saharan Africa, Latin America, Southeast Asia, and the Western Pacific [1].
Human infection with *T. solium* (taeniasis) typically occurs when pork containing viable cysticerci is consumed without sufficient cooking, allowing the larvae to mature into adult worms in the intestine [2]. Although many carriers do not report symptoms, some may experience nonspecific digestive complaints including abdominal discomfort, nausea, diarrhea, or bloating [2]. A different form of infection arises when *T. solium* eggs are ingested from contaminated food or water. This leads to cysticercosis, and when the central nervous system is affected,

neurocysticercosis develops—one of the leading causes of epilepsy acquired during adulthood in sub-Saharan Africa [3, 4]. The neurological consequences can manifest long after exposure and may involve seizures, headaches, hydrocephalus, stroke, and cognitive decline, with epilepsy occurring most frequently [5].

Pigs become infected when they ingest materials contaminated with human feces containing tapeworm eggs, which then give rise to cysts within muscle tissue and various organs [6]. Most infected animals appear clinically normal, yet extensive parasite loads can trigger muscle inflammation, reduced mobility, lethargy, disturbances in chewing, seizures, or even collapse [7, 8]. In addition to *T. solium*, pigs may also host *Taenia hydatigena*. Although not a zoonotic threat, its larvae grow within the membranes and organs of the abdominal and thoracic cavities, while dogs and other canids serve as hosts for the adult tapeworm [9].

Toxoplasma gondii is another globally distributed zoonotic parasite capable of infecting virtually all warm-blooded animals. Cats and other felids are the only hosts that shed the environmentally resistant oocysts, while pigs, humans, and many other species act as intermediate hosts [10]. Humans usually acquire toxoplasmosis by consuming undercooked meat containing tissue cysts or by accidentally ingesting oocysts present in soil, contaminated produce, or water [11]. Infections may also occur transplacentally, via organ transplantation or blood products, or through accidental exposure in laboratory settings [11]. While most postnatally acquired infections remain silent, congenital toxoplasmosis can result in miscarriage or stillbirth, and surviving infants may suffer from severe neurological or ocular disorders including hydrocephalus, retinochoroiditis, encephalitis, calcifications, or developmental impairments [12, 13].

Pigs acquire *T. gondii* in several ways: by ingesting oocysts contaminating the environment, by consuming infected rodents or birds, through cannibalism, or by vertical transmission [10]. Infection in pigs usually produces no visible symptoms but can occasionally cause pregnancy loss [14].

Epidemiological investigations conducted across numerous regions have consistently identified *T. solium* and *T. gondii* as highly prevalent parasites in many African countries [15–18]. Elevated infection rates in both pigs and humans are typically associated with settings where sanitation is poor, hygiene practices are limited, traditional free-range pig husbandry is common, and meat inspection systems are insufficient [1, 10]. In Burundi, pig rearing represents an important livelihood for low-income households, yet the conditions under which pigs are raised create multiple opportunities for the transmission of *Taenia* species and *T. gondii* [19]. Previous work in the country has documented *T. solium* cysticercosis prevalence rates of 15.5% in pigs based on tongue palpation and 31.5% in humans using antibody ELISA tests [19, 20]. Human exposure to *T. gondii* was reported at 44.1% using serological assays, although comparable data for pigs are still lacking [21].

Despite these earlier findings, updated estimates generated through more rigorous diagnostic approaches are necessary. Complete carcass dissection, using tissue slices no thicker than 5 mm, remains the reference standard for assessing *T. solium* infection, characterizing cysticerci, and evaluating infection intensity in endemic settings [22]. Partial carcass dissection—limited to tissues such as the heart, tongue, and masseter muscles—has been shown to perform reasonably well, with a reported sensitivity of about 81% [23]. Given the labor requirements and financial constraints associated with whole-carcass examination, partial dissection is generally recommended as a practical alternative for field studies [22, 23].

A comprehensive assessment of the presence and impact of *Taenia* spp. and *T. gondii* in pigs is essential to support evidence-based control strategies. Accordingly, the objectives of this study were (i) to determine the prevalence of *Taenia* spp. infections through meat inspection, partial carcass examination, and molecular verification, and (ii) to assess the seroprevalence of *T. gondii* in pigs.

Results

Slaughter facilities and characteristics of the sampled pigs

Among all slaughter slabs surveyed, only the national abattoir located in Bujumbura met basic operational and hygiene requirements, with appropriate infrastructure in place **(Figure 1)**. The remaining facilities—including the Gikoma slab in Bujumbura and those situated in Kayanza and Ngozi provinces—showed poor sanitary conditions. In these sites, pigs were often slaughtered outdoors on bare wooden or cement floors, and in some cases even in open bush areas **(Figure 2)**.

Across all sites, 576 pigs were examined. Females accounted for slightly more than half of the sample (320 of 576; 55.6%). The animals ranged in age from 6 to 36 months, with a mean age of 14 months. Regarding breed

composition, approximately 60% of the pigs were crossbreeds (predominantly Large White), while the remaining 40% were indigenous black pigs.

Figure 1. Pigs slaughtered at the national slaughterhouse in Bujumbura city

Figure 2. An example of a pig slaughter slab in the study area (Gikoma, Kayanza and Ngozi)

Prevalence of Taenia spp. identified through meat inspection, partial dissection, and molecular methods

Routine meat inspection detected cysticercosis in 14 of the 576 examined pigs, corresponding to a prevalence of 2.4% (95% CI: 1.3–4.0). When partial carcass dissection was applied, 67 pigs (11.6%, 95% CI: 9.1–14.5) were found to be infected (Table 1). Molecular testing confirmed *T. solium* in 66 of these 67 animals, giving an adjusted prevalence of 11.5% (95% CI: 9–14.4). Region-specific estimates showed infection rates of 9.0% in Bujumbura (26 pigs; 95% CI: 6–13), 16.7% in Kayanza (24 pigs; 95% CI: 11–23.8), and 11.1% in Ngozi (16 pigs; 95% CI: 6.5–17.4). Significant variation in infection prevalence was noted according to pig breed and location **(Table 1)**.

The number of cysticerci recovered from infected pigs varied markedly, ranging from a single cyst to 1,449, with a mean count of 80 per animal. The masseter muscles harbored the bulk of the cysts (76.1%), followed by the tongue (18.8%) and the heart (5.1%). The parasite load in the masseter muscles was significantly higher than in the other examined tissues ($p < 0.05$) **(Table 2)**. Overall, most cysticerci were viable (88.3%), whereas 6.4% were calcified and 5.3% showed degeneration **(Figures 3–5)**. Infection intensity did not differ significantly by province, sex, age, or breed **(Table 3)**.

Classifying pigs according to infection burden revealed that 68.7% (46 animals) had light infections, 13.4% (9 animals) exhibited moderate levels of infection, and 17.9% (12 animals) were heavily infected **(Table 4)**.

Table 1. Distribution of the prevalence of *Taenia solium* cysticercosis by meat inspection and partial carcass dissection

Variables	*N*	*P* MI	MI % (95% CI)	*P* PCD	PCD % (95% CI)	χ^2PCD	*p*-value
Provinces							
Bujumbura city	288	5	1.7 (0.6-4)	26	9.0 (6–13)	5.46	0.065
Kayanza	144	7	4.9 (2-9.8)	24	16.7 (11-23.8)		
Ngozi	144	2	1.4 (0.2–4.9)	17	11.8 (7.0-18.2)		
Slaughter slabs							
National slaughterhouse	144	1	0.7 (0.0-3.8)	14	9.7 (5.4–15.8)	5.93	0.431
Gikoma	144	4	2.8 (0.8-7)	12	8.3 (4.4–14.1)		
Kayanza	104	6	5.8 (2.2–12.1)	18	17.3 (10.6–26)		
Muhanga	40	1	2.5 (0.1–13.2)	6	15 (5.7–29.8)		
Ngozi	79	1	1.3 (0.0-6.9)	10	12.7 (6.2–22.1)		
Busiga	34	1	2.9 (0.1–15.3)	4	11.8 (3.3–27.5)		
Gashikanwa	31	0	0.0	3	9.7 (2-25.8)		
Sex							
Male	256	4	1.6 (0.4-4)	23	9 (5.8–13.2)	2.69	0.101
Female	320	10	3.1 (1.5–5.7)	44	13.8 (10.2–18)		
Age							
6–12 months	285	4	1.4 (0.4–3.6)	29	10.2 (6.9–14.3)	0.90	0.343
≥ 13 months	291	10	3.4 (1.7–6.2)	38	13.1 (9.4–17.5)		
Breed							
Local	228	9	4 (1.8–7.4)	39	17.1 (12.5–22.6)	10.14	0.001*
Crossed	348	5	1.4 (0.5–3.3)	28	8.1 (5.4–11.4)		
Origin of pigs							
Bujumbura	55	0	0.0	4	7.3 (2-17.6)	11.54	0.042*
Gitega	15	1	6.7 (0.2–32)	4	26.7 (7.8–55.1)		
Karusi	67	0	0.0	2	3 (0.4–10.4)		
Kayanza	221	8	3.6 (1.6-7.0)	31	14.0 (9.7–19.3)		
Kirundo	28	3	10.7 (2.3–28.2)	5	17.9 (6.1–36.9)		
Ngozi	190	2	1.1 (0.1–3.8)	21	11.1 (7-16.4)		
Total	576	14	2.4 (1.3-4.0)	67	11.6 (9.1–14.5)	-	-

N: Number of examined pigs, P: Number of infected pigs, MI: Meat inspection, PCD: Partial carcass dissection, CI: confidence interval, χ^2: chi-square, *significant

Table 2. *Taenia solium* cysticerci intensity and stages in organs and muscles

Organs and muscles	Infected pigs (%)	Viable	Degenerated	Calcified	Total	%	*p*-value
Heart	22 (32.8)	205	2	64	271	5.1	< 0.001 (Ref)
Tongue	39 (58.2)	891	28	82	1001	18.8	0.091
Masseter	40 (59.7)	3610	254	194	4058	76.1	< 0.001*
Total	-	4706 (88.3%)	284 (5.3%)	340 (6.4%)	5330	100	
Only tongue	20 (29.8)	24	0	5	29	0.6	
Only heart	6 (9)	2	0	5	7	0.1	
Only masseter	20 (29.8)	100	5	8	113	2.1	
Tongue + heart	1 (1.5)	0	0	5	5	0.1	

Tongue + masseter	5 (7.5)	591	228	53	872	16.4
Heart + masseter	2 (3)	23	0	5	28	0.5
Tongue + heart + masseter	13 (19.4)	3966	51	259	4276	80.2
Total	67 (100)	4706 (88.3%)	284 (5.3%)	340 (6.4%)	5330	100

*Significant

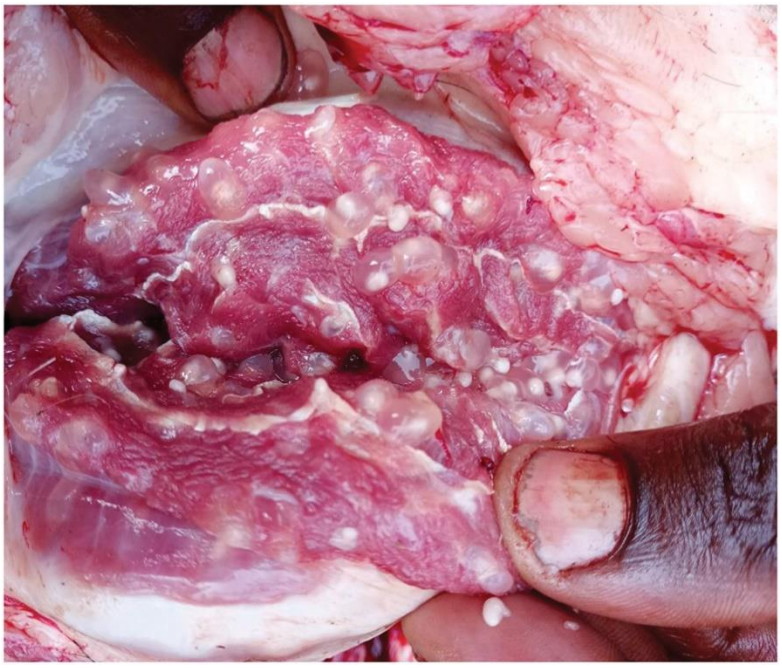

Figure 3. An example of a masseter muscle heavily infected with *Taenia solium* cysticerci

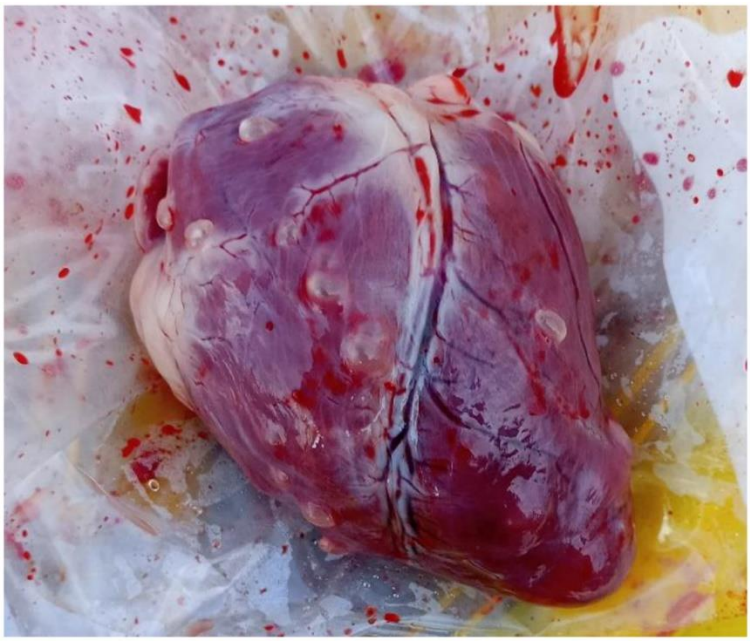

Figure 4. An example of a heart heavily infected with *Taenia solium* cysticerci

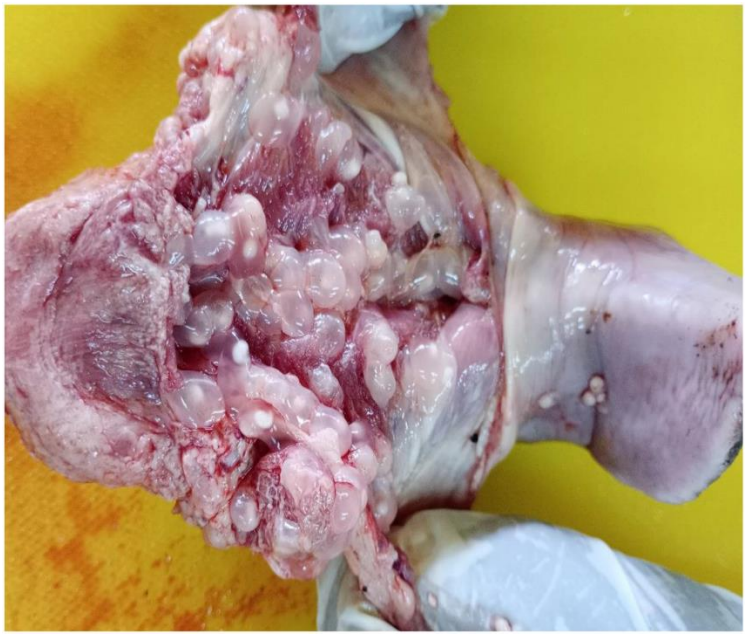

Figure 5. An example of a tongue heavily infected with *Taenia solium* cysticerci

Table 3. *Taenia solium* cysticerci intensity in infected pigs by province, sex, age and breed

Variables	Infected pigs	Total number and mean of cysticerci per infected pig	% of all cysticerci	*p*-value
Provinces				
Bujumbura city	26	1738 (66.8)	32.6	< 0.001 (Ref)
Kayanza	24	2809 (117)	52.7	0.317
Ngozi	17	783 (46.1)	14.7	0.546
Sex				
Female	44	3052 (69.4)	57.3	< 0.001 (Ref)
Male	23	2278 (99)	42.7	0.487
Age				
6–12 months	29	1353 (46.7)	25.4	< 0.001(Ref)
≥ 13 months	38	3977 (104.7)	74.6	0.097
Breed				
Crossed	28	1708 (61)	32.0	< 0.001 (Ref)
Local	39	3622 (92.9)	68	0.394

Table 4. Proportion of *Taenia solium* infection levels in infected pigs per province

Infection intensity level	Bujumbura city	Kayanza	Ngozi	Total	%	Mean of cysticerci per infected pig
Light (1–10 cysticerci)	19	13	14	46	68.7	2
Moderate (11–100 cysticerci)	3	6	0	9	13.4	49
Heavy (101–1449 cysticerci)	4	5	3	12	17.9	402
Total	26	24	17	67	100	80
Light (diagnosed by MI)	0	0	0	0/46	0.00	-
Moderate (diagnosed by MI)	1	3	0	4/9	44.4	-
Heavy (diagnosed by MI)	4	4	2	10/12	83.3	-
Total	5	7	2	14/67	20.9	-

MI: Meat inspection

A total of 576 pigs were assessed for lesions attributable to *Taenia hydatigena*. Evidence suggestive of this parasite was found in 32 animals (5.5%, 95% CI: 3.8–7.8). The clinical appearance of these cases varied: some pigs

displayed conspicuous cysts in the liver or mesentery, whereas others had only small nodules or minor hepatic alterations. Subsequent molecular testing clarified the nature of these findings. Twenty-four of the suspected cases, including all pigs presenting the larger abdominal cysts and most animals with the smaller liver lesions, were verified to be infected with *T. hydatigena*. One pig with a minute hepatic cystic structure was instead identified as harboring *T. solium*, consistent with its positive results from the partial carcass dissection and molecular assays conducted earlier.

When restricting the analysis to laboratory-confirmed infections, the prevalence of *T. hydatigena* was 4.2% (95% CI: 2.7–6.1). This proportion was essentially the same in all three surveyed locations—Bujumbura city as well as Kayanza and Ngozi provinces each recorded the same infection percentage. Mixed infections were uncommon, although two pigs carried both *T. solium* and *T. hydatigena*: in one animal, cysticerci of the two species occupied different tissues within the head and abdomen, and in the other, tongue lesions due to *T. solium* coincided with *T. hydatigena* cysts in the liver. Seven additional liver samples that initially appeared suspicious but yielded negative PCR-RFLP results were further examined using multiplex PCR and were confirmed to be free of *Echinococcus* and *Sarcocystis* species.

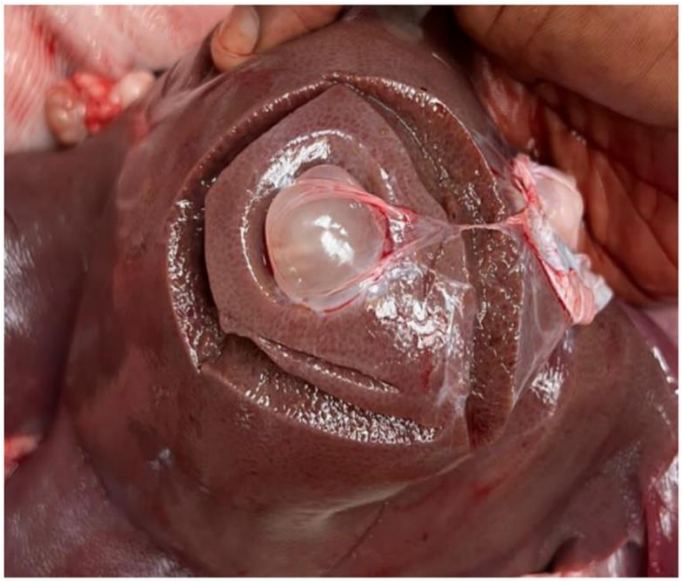

Figure 6. Liver infected with *Taenia hydatigena* cysticerci

Figure 7. Large *Taenia hydatigena* cysticerci found in the abdominal cavity (mesentery)

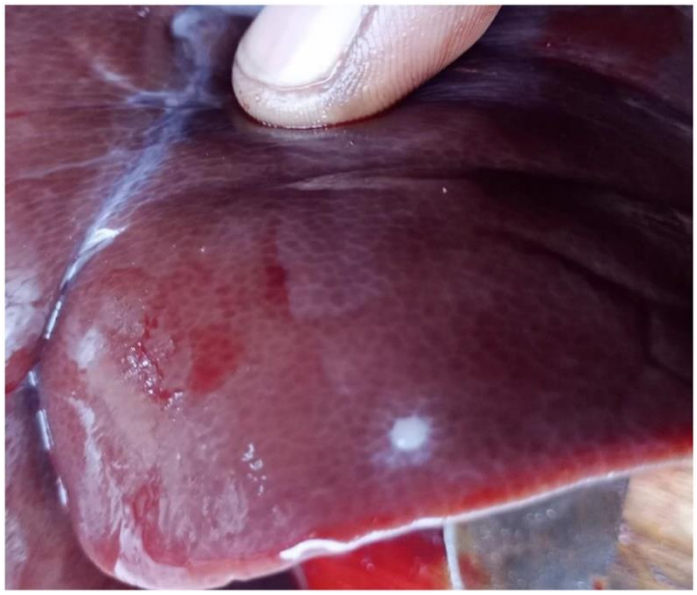

Figure 8. Liver with a suspected lesion (small white nodule)

Prevalence of T. gondii infection in pigs based on serological analyses

Out of the 576 pig serum samples analyzed, 102 animals tested positive for antibodies against *Toxoplasma gondii*, corresponding to a seroprevalence of 17.7% (95% CI: 14.7–21.1) **(Table 5)**. Statistical analysis revealed that seropositivity was significantly influenced by the pigs' sex, the location of the slaughter slabs, and the geographic origin of the animals **(Table 5)**.

Table 5. Distribution of the prevalence of toxoplasmosis in pigs in Burundi using indirect ELISA

Variables	Examined pigs	Infected pigs	Prevalence % (95% CI)	Chi-square	*p*-value
Provinces					
Bujumbura city	288	52	18.1 (13.9–23)	0.43	0.807
Kayanza	144	23	16 (10.4–23)		
Ngozi	144	27	18.8 (12.7–26.1)		
Slaughter slabs					
National slaughterhouse	144	17	11.8 (7.0-18.2)	16.45	0.012*
Gikoma	144	35	24.3 (17.6–32.2)		
Kayanza	104	15	14.4 (8.3–22.7)		
Muhanga	40	8	20 (9.1–35.7)		
Ngozi	79	21	26.6 (17.3–37.7)		
Busiga	34	4	11.8 (3.3–27.5)		
Gashikanwa	31	2	6.5 (0.8–21.4)		
Sex					
Male	256	35	13.7 (9.7–18.5)	4.67	0.031*
Female	320	67	20.9 (16.6–25.8)		
Age					
6–12 months	285	48	16.8 (12.7–21.7)	0.18	0.667
≥ 13 months	291	54	18.6 (14.3–23.5)		
Breed					
Local	228	44	19.3 (14.4–25.0)	0.49	0.486
Crossed	348	58	16.7 (12.9–21.0)		
Origin of pigs					
Bujumbura	55	20	36.4 (23.8–50.4)	23.76	< 0.001*
Gitega	15	1	6.7 (0.2–32)		
Karusi	67	4	6 (1.7–14.6)		
Kayanza	221	32	14.5 (10.1–19.8)		
Kirundo	28	5	17.9 (6.1–36.9)		
Ngozi	190	40	21.1 (15.5–27.5)		

Total	576	102	17.7 (14.7–21.1)	-	-

CI: Confidence interval, *significant

This study provides the first evidence of *Taenia hydatigena* and *Toxoplasma gondii* in pigs in Burundi. Notably, the prevalence of *T. solium* cysticercosis estimated through partial carcass dissection was nearly five times higher than that detected via routine meat inspection. This discrepancy can be attributed to the higher sensitivity of partial dissection (approximately 81%) compared with meat inspection (22.1%), particularly for light infections, which comprised the majority of cases in our study (68.7%) [23, 24]. Routine meat inspection in Burundi primarily identifies only moderate to heavy infections, overlooking most light infections. Such undetected infections pose a public health concern because the majority of cysticerci observed were viable, meaning infected pork entering the food chain could transmit *T. solium* to consumers.

The prevalence detected through meat inspection in this study was low, aligning with findings from Kenya (1.8%), India (1.4%), Uganda (0.6%), Madagascar (4.6%), and South Africa (5%) [25–29]. One factor contributing to the low prevalence in Burundi is the practice of tongue palpation prior to formal sale or slaughter. This often results in clandestine or home slaughter of pigs identified as infected, a strategy used to avoid carcass condemnation [19]. Previous farm-based studies in Burundi reported *T. solium* cysticercosis prevalence of 15.5% in pigs using tongue palpation [19]. Despite its low sensitivity for light infections (around 21%) [24], tongue palpation remains a rapid and inexpensive tool that traders and butchers use to select pigs deemed healthy for slaughter.

However, this practice carries economic and public health implications. Pigs found to be infected through tongue palpation experience a sharp reduction in market value, sometimes up to 80% [19, 30], incentivizing farmers to slaughter infected pigs at home for family or neighborhood consumption or sell them clandestinely through informal channels. Such practices facilitate the persistence of *T. solium* transmission in the community: consumers, often local farmers themselves, may become infected with adult pork tapeworms, thereby increasing environmental contamination with *Taenia* eggs and perpetuating the cycle of infection in both pigs and humans.

Partial carcass dissection was employed in this study as an alternative to full carcass dissection—the recognized gold standard—for estimating the intensity and developmental stages of *T. solium* cysticerci in pig muscles and organs [23]. Previous studies using partial dissection of the tongue, heart, and masseter muscles have reported similar prevalence rates, such as 12.1% in Peru and 17.6% in Cameroon [23]. With an estimated sensitivity of 81%, this method offers several advantages under research conditions, including reduced costs when sampling large numbers of pigs, as well as less labor and time compared to full dissection [22, 23]. Despite its higher sensitivity relative to standard meat inspection, routine implementation at slaughter slabs is impractical because it is labor-intensive, time-consuming, and results in the partial loss of meat value, in addition to potentially limited familiarity with the technique among inspectors.

The present findings revealed that local breed pigs exhibited higher infection rates than crossbreeds, and pigs originating from Gitega and Kirundo provinces were more affected than those from other areas. This disparity is likely linked to differences in farming practices: crossbreeds are predominantly raised on larger farms with improved management, including regular deworming and commercial feed, whereas local black pigs are often reared on small-scale farms under traditional, free-ranging systems. In densely populated provinces such as Gitega, Kirundo, Kayanza, Muyinga, and Ngozi, pigs are mostly kept on smallholder farms using low-input methods aimed at household income generation [19, 31]. These conditions increase the likelihood of pigs encountering *T. solium* eggs in the environment.

Regarding infection intensity, 65.4% of infected pigs in Tanzania and 76% in Zambia had light to moderate infections, consistent with the current observations [22, 32]. Free-range pigs are more prone to moderate or heavy infections, often due to ingesting human feces contaminated with high concentrations of tapeworm eggs during open defecation [33]. In contrast, light infections may result from limited exposure, such as consuming small numbers of eggs from the environment, contaminated water, or vegetation brought into pig pens [33]. These dynamics explain the three observed infection levels in Burundi, corresponding to fully penned pigs, partially penned pigs, and free-ranging pigs [19].

The distribution of cysticerci within the carcass also mirrors findings from Cameroon, where masseter muscles harbored more cysts than the tongue or heart [34]. Given the high prevalence and intensity of cysticerci in the masseter and tongue muscles, the Ministry responsible for livestock should consider enforcing inspection of these tissues, which are currently excluded from routine meat inspection policies [35]. Alarmingly, in this study, pigs with light and moderate infections—and many with heavy infections—entered the food chain. This situation partly

reflects regulatory gaps that allow pigs with subclinical infections to be slaughtered for consumption, combined with lapses in vigilance by meat inspectors [35]. Consequently, there is a need for rigorous enforcement of inspection protocols and careful handling of carcasses, especially those with light infections that may go undetected, as they can still pose a risk for human taeniasis [33].

To mitigate these risks, it is essential to raise awareness among consumers about safe culinary and consumption practices. Complementary strategies should include educational campaigns promoting improved pig husbandry, hygiene and sanitation measures, and treatment of human tapeworm carriers, all of which are critical for reducing *T. solium* transmission between pigs and humans [36].

Molecular testing revealed that the non-zoonotic tapeworm *Taenia hydatigena* is present in pigs in Burundi, with an overall prevalence of 4.2%. This is similar to findings reported elsewhere in Africa [37]. The occurrence of *T. hydatigena* in pigs likely reflects infections in local dog populations, as dogs can acquire the parasite by consuming infected offal from slaughter slabs or home slaughter, and subsequently contaminate the environment with eggs through their feces [9]. Co-infections involving both *T. hydatigena* and *T. solium* have been documented in countries such as Tanzania, Cameroon, and Zambia, highlighting the potential for multiple tapeworm species to circulate in pig-rearing communities [22, 34, 38]. To reduce transmission, careful management of slaughter by-products that may harbor cysticerci, combined with improvements in pig-rearing practices, is recommended.

The study also confirmed exposure of Burundian pigs to *Toxoplasma gondii*. The seroprevalence observed is within the range commonly reported across Africa, where rates typically vary between 17% and 34% and average around 25% [39]. Higher prevalence has been documented in Kenya, Ethiopia, South Africa, and Ghana [40–43], while studies from South America and Asia generally report comparable or slightly higher rates. In contrast, European countries often show lower seroprevalence [39]. Differences between regions are likely linked to husbandry practices, environmental conditions such as temperature and rainfall, farm hygiene, and the presence of cats and rodents that facilitate parasite transmission [10].

Although the indirect ELISA used in this study effectively detects antibodies against the *T. gondii* P30 protein and offers high sensitivity and specificity [44, 45], it cannot distinguish between past and active infections, nor does it indicate whether cysts in tissues are viable. No cross-reactions with other apicomplexan parasites have been reported for this assay in pigs [44, 45]. Pigs raised under free-range conditions are at higher risk of infection due to exposure to sporulated oocysts in the environment, consumption of infected rodents or birds, or contaminated water [10, 46]. In Burundi, the common practice of keeping cats for rodent control—often allowing them access to pig pens, feed, and water—likely contributes to transmission even in penned pigs. Evidence from confined pigs in the United States, which show less than 1% seroprevalence when cats and rodents are excluded, underscores the importance of restricting such contacts to prevent infection [47].

As a zoonotic agent, *T. gondii* in pigs poses a potential risk to humans, particularly if pork is consumed undercooked. Routine meat inspection cannot detect tissue cysts, meaning infected meat may enter the food chain unnoticed [48]. Proper cooking of pork—frying, roasting, or heating to at least 67 °C for three minutes—is necessary to inactivate cysts [49]. Public health interventions should focus on educating communities about safe pig management, cat control, hygiene, sanitation, and proper pork preparation. These measures not only help prevent human toxoplasmosis but also reduce exposure to *T. solium* cysticercosis.

Conclusions

The results of this study confirm that *Taenia solium* and *Toxoplasma gondii*, two significant foodborne parasites, are present in pig populations in Burundi. Their occurrence highlights potential health risks for the local population, particularly through consumption of pork contaminated with these pathogens. It is possible that the prevalence of *T. solium* cysticercosis reported here underestimates the true burden, as pigs detected with cysticerci during tongue palpation at farms may not enter formal slaughter facilities, and partial carcass dissection, while sensitive, does not detect all infections. To limit ongoing transmission to both pigs and humans, comprehensive control measures are warranted. Recommended strategies include improved pig management, enhanced hygiene and sanitation, strengthened meat inspection and monitoring of infected animals, safe cooking practices, and treatment of human tapeworm carriers.

Methods

Study area

The investigation was carried out in slaughter facilities located in both urban and rural regions of Burundi, including Bujumbura city and the provinces of Ngozi and Kayanza **(Figure 9)**. Bujumbura, the country's economic capital, was selected due to its role as a major hub for pigs sourced from surrounding rural areas. The provinces of Kayanza and Ngozi were included because of their high population density and the prevalence of extensive pig-rearing systems, which provide a significant source of income for smallholder farmers [50].

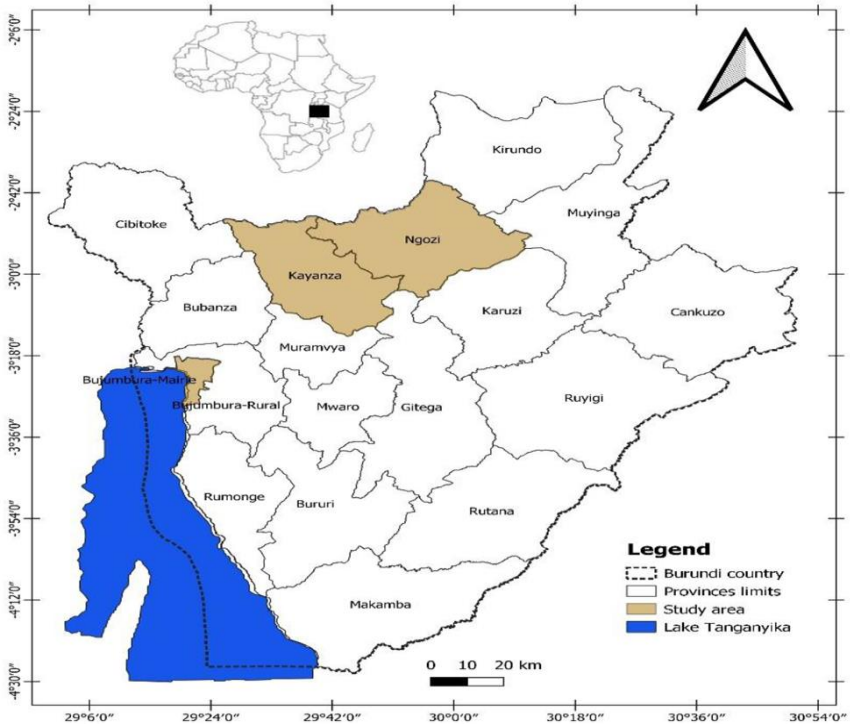

Figure 9. Map of Burundi showing the study area

Study design and sample collection

A cross-sectional investigation combining on-site observations and laboratory analyses was carried out from October 2023 to March 2024. Slaughter facilities were selected based on the daily number of pigs processed. The study included the national slaughterhouse in Bujumbura, the Gikoma slaughter slab within Bujumbura city, several slaughter slabs in Kayanza and Muhanga communes (Kayanza province), and facilities in Ngozi, Gashikanwa, and Busiga communes (Ngozi province). On days when more than ten pigs were available, animals were chosen randomly; when fewer than ten pigs were present, all eligible pigs were sampled. Selection criteria included apparent health, age over five months, sex, breed, and geographic origin.

Sample size calculations followed the formula $N = Z2pq/L2$ [51]. taking into account a previously reported *T. solium* prevalence of 15.5% in Burundi based on tongue palpation [19] and an African porcine *T. gondii* seroprevalence of 25% [39]. Since both infections were studied in the same population, the minimum sample size was 288 pigs. To ensure representation from urban and rural settings, the study ultimately sampled 576 pigs: 288 from Bujumbura city and 288 from Kayanza and Ngozi provinces.

On-Site Data Collection and Sample Handling

Participating pigs were provided by traders and butchers who consented to the study. Basic information, including age, sex, breed, and origin, was recorded for each animal. Blood samples were drawn from the jugular vein into 50 mL Falcon tubes immediately after slaughter. Meat inspections were performed by veterinarians following national guidelines [35]. These procedures include systematic incisions of the thigh, abdominal wall, psoas, diaphragm, intercostal muscles, larynx, and heart to detect *T. solium* cysticerci [35]. In some slabs, inspectors extended these incisions to fore- and hind-limb muscles and the heart to enhance detection, whereas at the Bujumbura national slaughterhouse, deep cuts were consistently made in the forelimb muscles, heart, and tongue of every carcass.

During carcass examination, the liver and mesentery were carefully inspected for *T. hydatigena* cysts. Large cysts (≥2 cm), translucent and fluid-filled with a distinct white spot representing the scolex, were classified as *T.*

hydatigena. Smaller cysts or ambiguous liver lesions were recorded as suspected cysticerci, possibly belonging to *T. hydatigena* or *T. solium*. Large cysts were preserved in 50 mL Falcon tubes containing 70% ethanol, while smaller lesions were stored in 2 mL cryovials with ethanol for molecular analyses.

After meat inspection, samples of the tongue, heart, and masseter muscles were purchased from traders for further testing. All blood and meat specimens were labeled, maintained in cool boxes, and transported to the National Veterinary Laboratory in Bujumbura for serological testing and partial carcass dissection. Subsamples intended for molecular analysis were sent to the Institute of Tropical Medicine in Antwerp, Belgium, and the Laboratory of Foodborne Parasitic Zoonoses at Ghent University, Belgium. Results from meat inspection, partial carcass dissection, and molecular assays were systematically documented for each pig.

Laboratory analyses

Partial carcass examination Selected muscles (tongue, heart, and masseter) were cut into slices <5 mm thick and systematically searched for *T. solium* cysticerci [52]. All detected cysticerci were counted and grouped macroscopically into three categories: viable (clear vesicular fluid containing a visible white protoscolex), degenerated (altered or opaque cyst wall, or no fluid present), or calcified (firm, cheese-like content) [22]. The degree of infection was defined as light (1–10 cysts), moderate (11–100 cysts), or heavy (>100 cysts). From each positive carcass, a maximum of five cysts recovered from these three muscle groups were pooled and fixed in 2-mL cryovials with 70% ethanol for subsequent molecular identification.

Serological testing Blood samples were kept overnight at 4 °C to allow clot formation. Serum was then separated, divided into aliquots in 2-mL cryovials, and frozen at –20 °C until use. Detection of anti-*Toxoplasma gondii* antibodies was performed using a commercial indirect multi-species ELISA targeting the P30 surface antigen (ID Screen® Toxoplasmosis Indirect Multi-species), strictly according to the manufacturer's instructions.

Molecular identification Cysticerci and any suspicious lesions were shipped to the Helminthology Laboratory, Institute of Tropical Medicine, Antwerp, Belgium, for confirmatory testing. *Taenia* species differentiation was carried out by PCR-RFLP. Total genomic DNA was extracted with the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) following the recommended protocol. A portion of the mitochondrial 12S rDNA gene was amplified using the primer pair ITM TnR-TaenF and nTAE [55]. Amplicons were digested with the restriction endonucleases DdeI, HinfI, and HpaI, and the resulting fragment patterns were compared to reference profiles for *T. solium* and *T. hydatigena* [53, 54]. Samples from liver lesions that tested negative by PCR-RFLP were further screened by multiplex PCR [55] for *Echinococcus* spp. and *Sarcocystis* spp. at the Laboratory of Foodborne Parasitic Zoonoses, Ghent University, Belgium.

Statistical analysis

Field and laboratory findings were first captured in Microsoft Excel, then transferred to R (version 4.3.3) for all computations [56]. Summary measures such as absolute and relative frequencies, means, proportions, and their corresponding 95% confidence intervals were calculated. Relationships between cysticercosis prevalence and categorical predictors (province, age category, sex, breed, and source of pigs) were tested with the χ^2 test (or Fisher's exact test when appropriate).

To model the number of cysticerci recovered from the tongue, heart, and masseter muscles, count data regression was applied: Poisson models were initially fitted, and negative binomial models were used when overdispersion was present. In both approaches, age, sex, breed, and province were entered as explanatory variables. Statistical significance was declared at $p < 0.05$.

Abbreviations

CI	Confidence interval
ELISA	enzyme-linked immunosorbent assay
MI	Meat inspection
PCD	Partial carcass dissection
PCR RFLP	Polymerase chain reaction-restriction fragment length polymorphism

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Conflict of Interest: None

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Ethics Statement: The research protocol was approved by the Institutional Review Board of the Institute of Tropical Medicine (ITM) in Belgium (IRB/RR/AC/091 Ref 1595/22) and the National Ethics Committee in Burundi (CNE/25/2022). Permission and verbal consent were obtained from local administration, slaughter slab managers, butchers, and pig traders to collect pig blood and meat samples at the slaughter slabs.

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