งานประชุมวิชาการ
สถาบันสุขภาพสัตว์แห่งชาติ ประจำปี พ.ศ. 2569

Hidden Zoonotic Risk in Cardiac Sarcocystosis of Cattle: Integrative Histopathological and Molecular Evidence from Upper Northern Thailand

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21-23 กรกฎาคม 2569
โรงแรมเฟลิกซ์ ริเวอร์ แคว รีสอร์ท จ.กาญจนบุรี

Our outlines

Introduction
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Methodology
Our results
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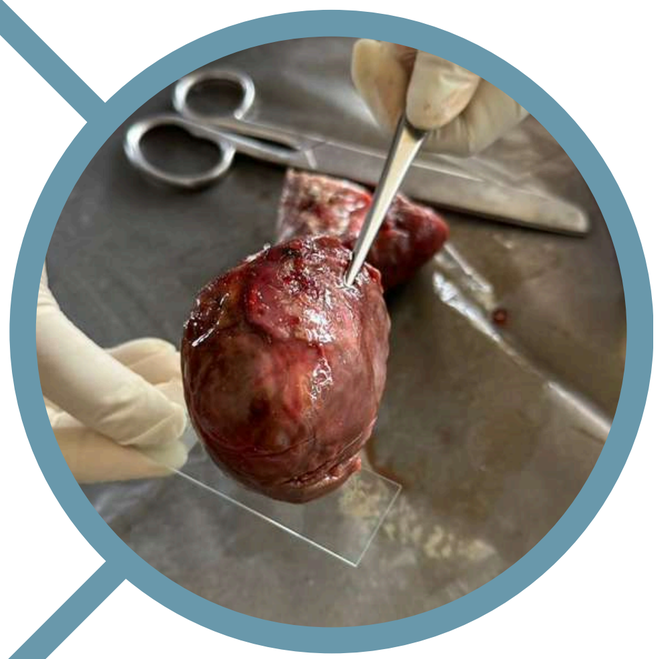
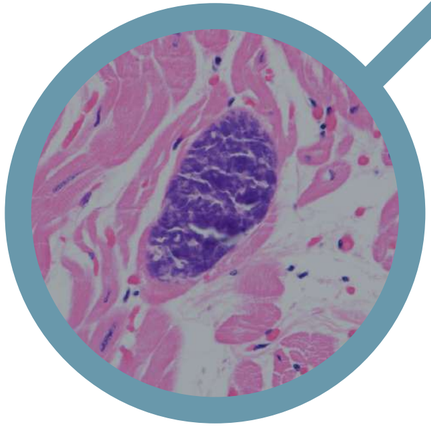
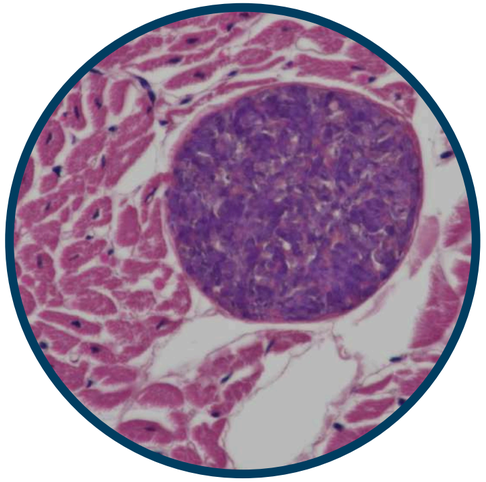




Introduction

Sarcocystosis

- Sarcocystosis is a globally distributed zoonotic parasitic disease caused by intracellular protozoan parasites belonging to the genus Sarcocystis.
- A two-host heteroxenous life cycle based on a predator-prey relationship.
- Ingestion of tissue cysts leads to temporary gastrointestinal symptoms in definitive hosts (carnivores/predators), while ingestion of environmental sporocysts can cause severe systemic illness and muscle pathology in intermediate hosts (herbivores/prey)

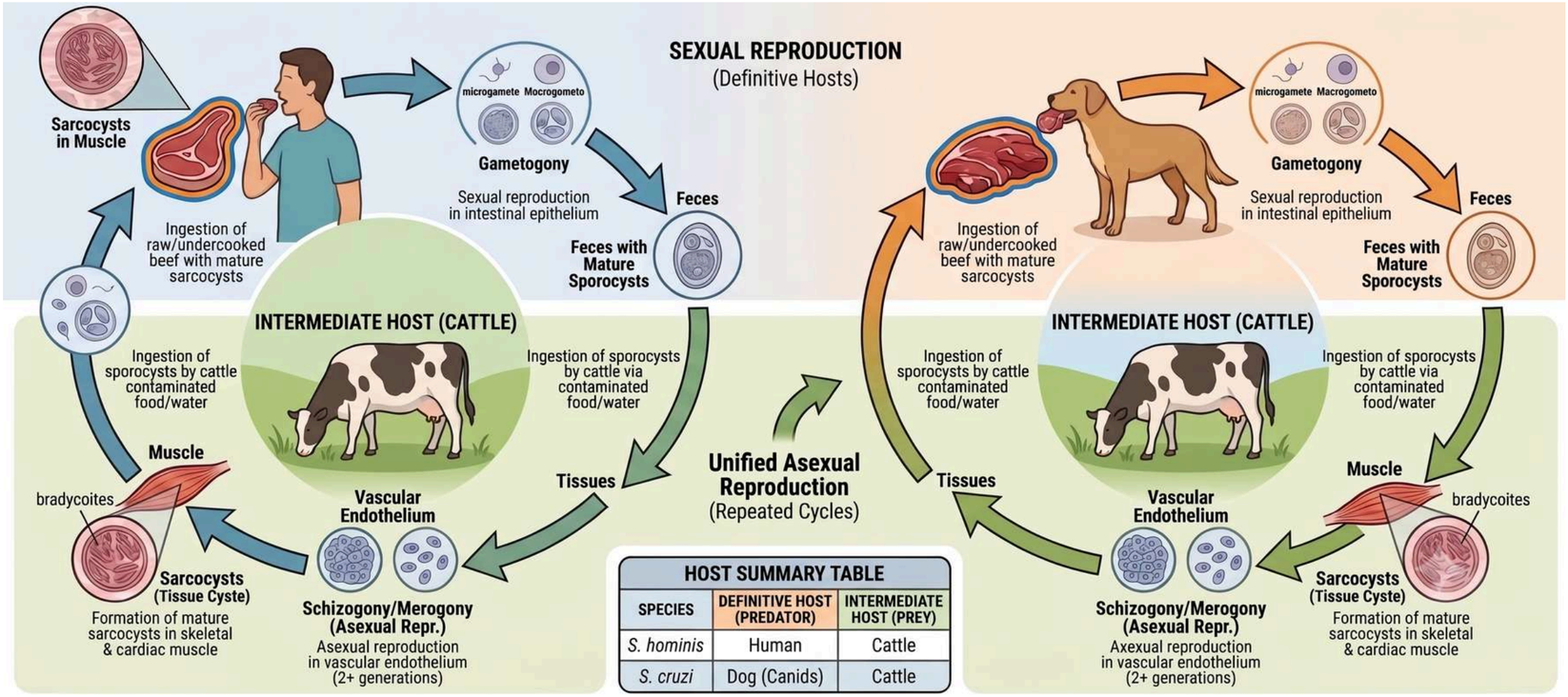


Phylum: **Apicomplexa**
Family: **Sarcocystidae**
Subfamily: **Sarcocystinae**
Genus: **Sarcocystis**

Target Species

Sarcocystis hominis
Definitive host: **humans**,
Intermediate host: **cattle**

Sarcocystis cruzi
Definitive host: **canids/dogs**,
Intermediate host: **cattle**



Introduction

Livestock impact

- Predominantly subclinical cases: Most infections are chronic and asymptomatic, with tissue cysts persisting quietly in the host over long periods without causing visible disease.
- Potential for acute disease: Severe clinical cases (fever, anemia, drops in milk yield) are less common and typically occur only under heavy, acute environmental exposure to *S. cruzi.*
- Economic impact: The primary livestock burden is economic, driven by sporadic protozoal abortions and down-grading/rejection of carcasses at the slaughterhouse due to muscle discoloration (lesion from cyst).

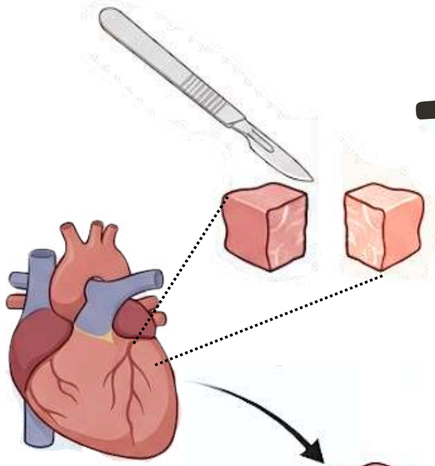
Public health impact

- Zoonotic food safety concern: Intestinal sarcocystosis (*S. hominis*) is a foodborne public health risk driven by human consumption of raw or undercooked beef containing tissue cysts. (intermediate host for both predators)
- Cultural dietary risk factors: Exposure risks are significantly amplified by regional culinary traditions, specifically the popularity of local raw beef delicacies in Thailand.
- High historical detection rate: Historical data confirm its presence of the parasite in the region, with historical stool screening programs showing detection rates of up to 23.2% of Sarcocystis among 362 asymptomatic Thai laborers, peaking at 26.6% in some areas.
- The definitive diagnosis requires the identification of oocysts/sporocysts in a patient’s faeces, even a healthy adult can be infected. **Intestinal sarcocystosis** nausea, vomiting, stomachache, diarrhea, and acute or chronic enteritis. (many cases are mild or asymptomatic)

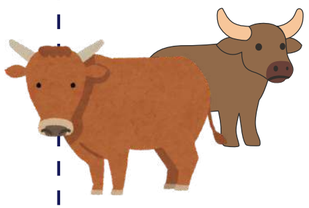
Objectives

- To investigate *Sarcocystis spp*. infection in cardiac tissue from livestock, with emphasis on histopathological characterization and molecular species identification in upper northern Thailand.

Methodology



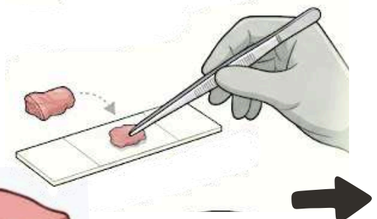
298 Heart samples
Sep 2023 -Mar 2026



No autolysis
Good quality
and quantity samples

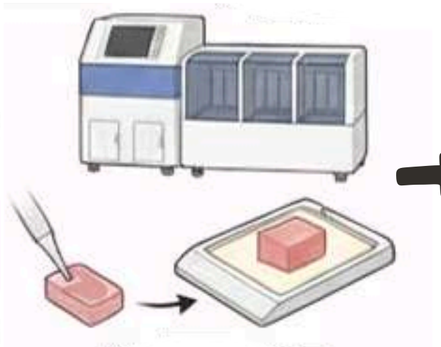
10% buffer formalin

Parasitology method

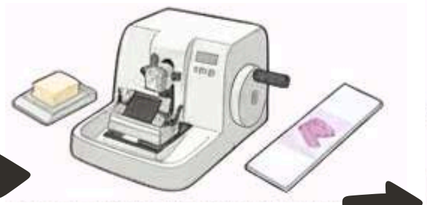


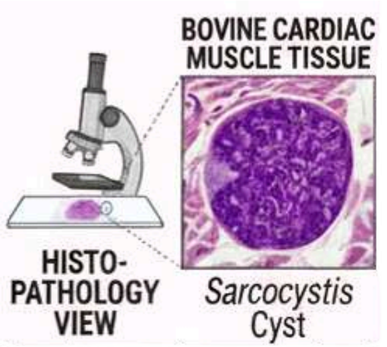
Tissue stamp or tissue impression smear

Pathology method



Tissue processing and paraffin embedding

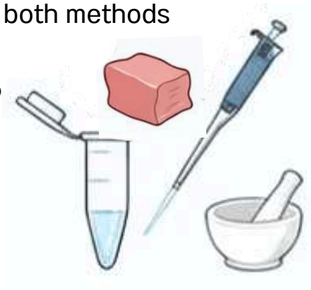




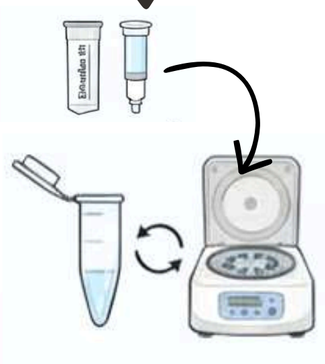
- HE staining
- Periodic acid-Schiff (PAS)
- Masson's trichrome
- TEM

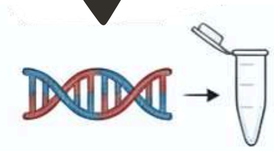
Positive case; cyst should be clear, measurable and represent cysts more than 3 LPF at 10X Objective

Positive case from both methods



DNA extraction



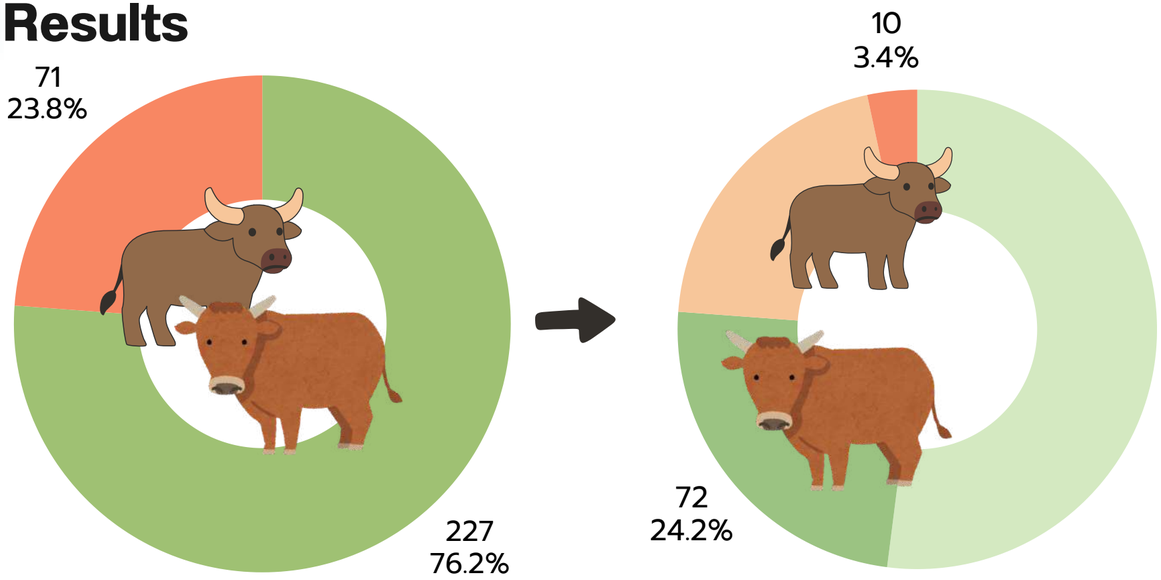


PCR assay, 18S rRNA for species identification
Sequencing

*All samples were tested negative for BRDC, *Pasteurella multocida, Mannheimia haemolytica*, *Klebsiella pneumoniae*

Chiesa *et al.*, 2013

Results



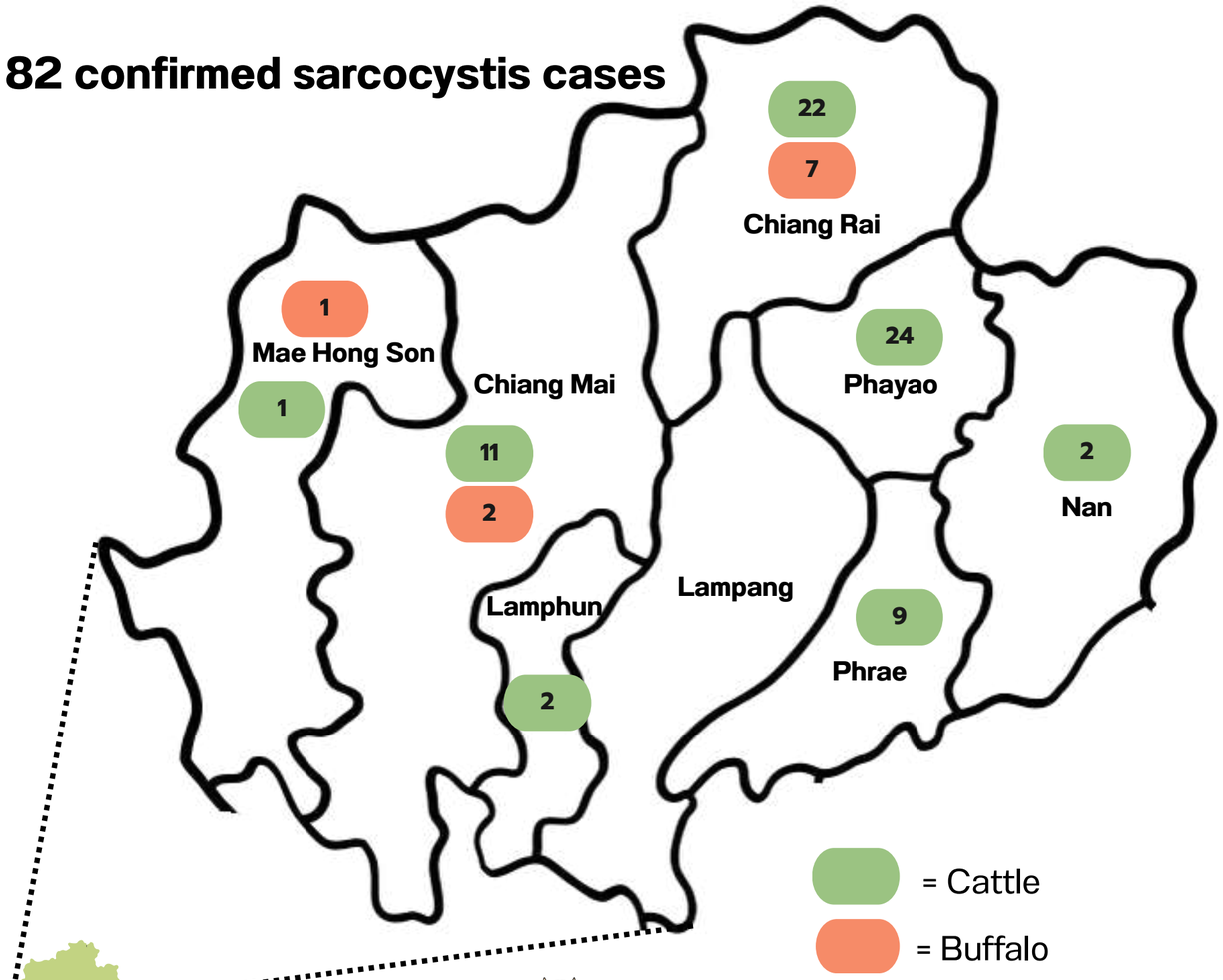
298 examined samples

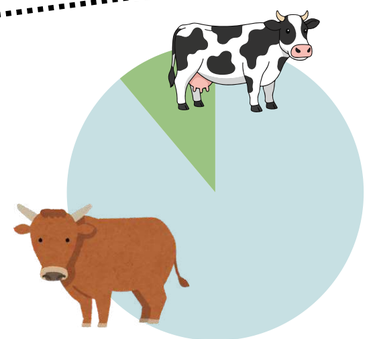
Sarcocysts were detected in 82 samples from both methods

- 82 samples (27.5%) were confirmed positive for Sarcocysts.
- Cattle: 72 out of 227 examined (31.7%)
- Buffalo: 10 out of 71 examined (14.1%)

- Positive cattle cases (72) were detected across 7 of the 8 provinces surveyed. Conversely, positive buffalo cases (10) were identified in only three specific provinces: Chiang Rai (7), Chiang Mai (2), and Mae Hong Son (1).
- Phayao (24 cattle cases) and Chiang Rai (22 cattle, 7 buffalo cases) act as major zones, accounting for 64.6% (53 out of 82) of all positive cases detected.

Provincial mapping of the 82 confirmed sarcocystis cases



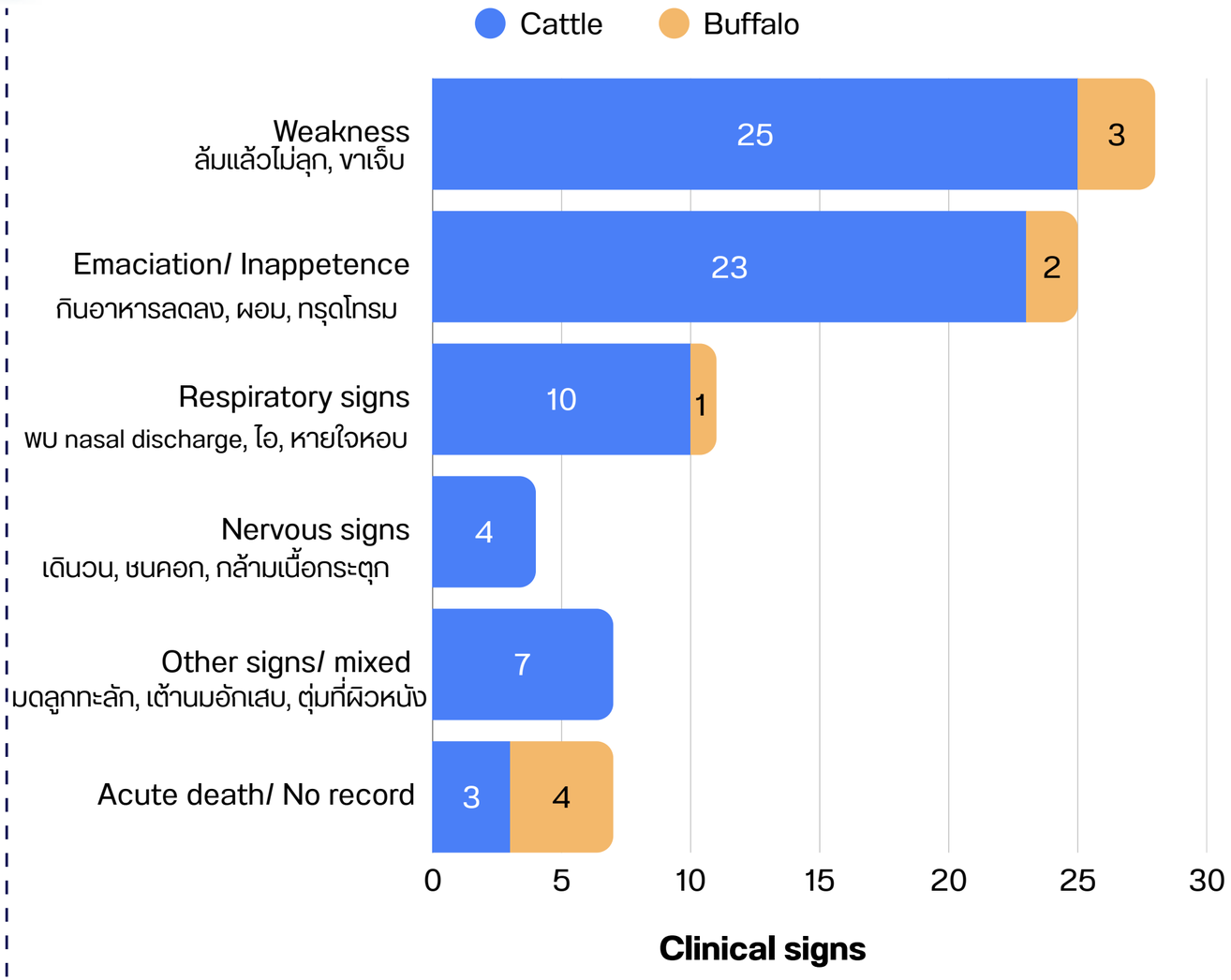


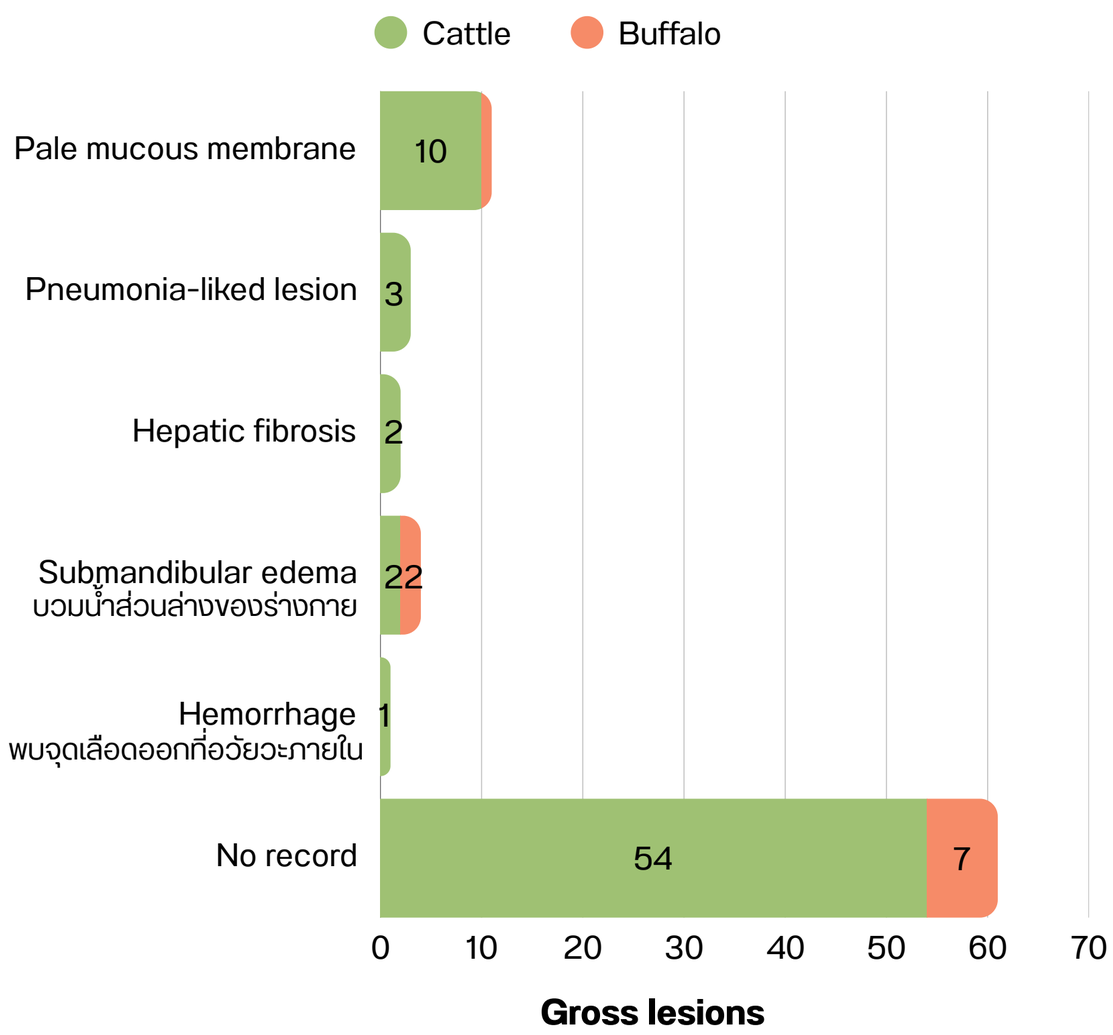
Beef cattle 64/72 (89%)
Dairy cattle 8/72 (11%)

	Submitted cases		Positive cases	
	Cattle	Buffalo	Cattle	Buffalo
Chiang Rai	60	21	22	7
Phayao	71	23	24	-
Chiang Mai	22	9	11	2
Lamphun	19	1	2	-
Phrae	36	5	9	-
Nan	8	5	2	-
Mae Hong Son	4	3	1	1
Lampang	7	4	-	-
Total	227	71	72	10

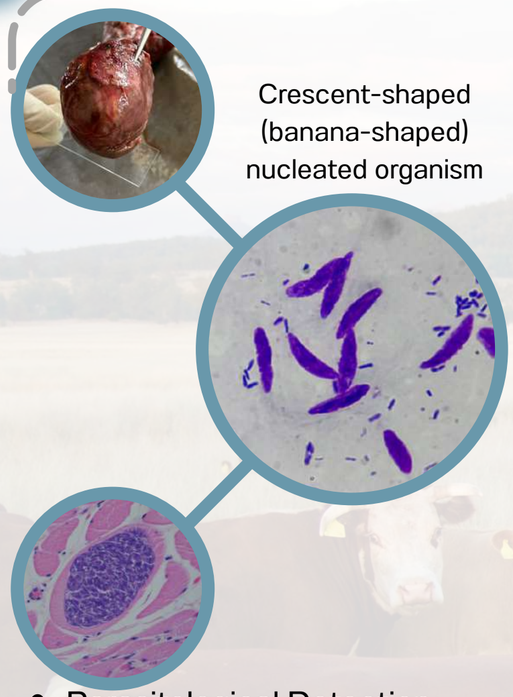
Results

Clinical and pathological profiles of the 82 confirmed sarcocystis cases





Results



Crescent-shaped (banana-shaped) nucleated organism

- Parasitological Detection: Outlines the initial parasitology screen step.
- Histopathological Confirmation (H&E on Cardiac Tissue): Highlights the specific 10X low-power field (LPF) threshold requirement (at least 3 LPF areas with detected cysts).

Profiles and lesion of 17 selected cases

Species	Sex	Age (Y)	Sample type	Location	Clinical signs	Gross lesions
Dairy cattle	F	8	Tissues	Mueang, Chiang Mai	Loss of appetite/Dyspnea	NA
Dairy cattle	F	3.4	Tissues	San Pa Tong, Chiang Mai	Loss of appetite	NA
Dairy cattle	F	8	Tissues	San Pa Tong, Chiang Mai	Loss of appetite/Depress	NA
Cattle	F	11	Tissues	Mueang, Phrae	Emaciation	NA
Cattle	F	11.5	Tissues	Mueang, Phrae	Emaciation/Depress	NA
Cattle	F	9	Tissues	Mueang, Phrae	Weakness/Depress	NA
Cattle	F	9	Tissues	Mueang, Phrae	Weakness/Depress	NA
Cattle	F	11	Tissues	Mueang, Phrae	Weakness/Depress	NA
Cattle	F	NA	Tissues	Mae Suai, Chiang Rai	NA	Pale mucous membrane, Pulmonary edema
Cattle	F	4.6	Tissues	Wiang Chiang Rung, Chiang Rai	Respiratory sign	NA
Cattle	F	4	Tissues	Pan, Chiang Rai	Weakness/Depress/Fever	NA
Cattle	F	10	Tissues	Mueang, Chiang Rai	NA	NA
Cattle	F	2.4	Tissues	Phu Kamyao, Phayao	Loss of appetite/Depress/ Epistaxis	NA
Cattle	F	2	Tissues	Phu Kamyao, Phayao	Dyspnea	Petechial hemorrhages at multipleorgans
Cattle	F	4	Tissues	Phu Kamyao, Phayao	Weakness/sternal recumbency	NA
Cattle	F	11.1	Tissues	Mueang, Phayao	Weakness/sternal recumbency	NA
Cattle	F	10	Tissues	Dok Khamtai, Phayao	Weakness	Petechial hemorrhages at omentum

Results

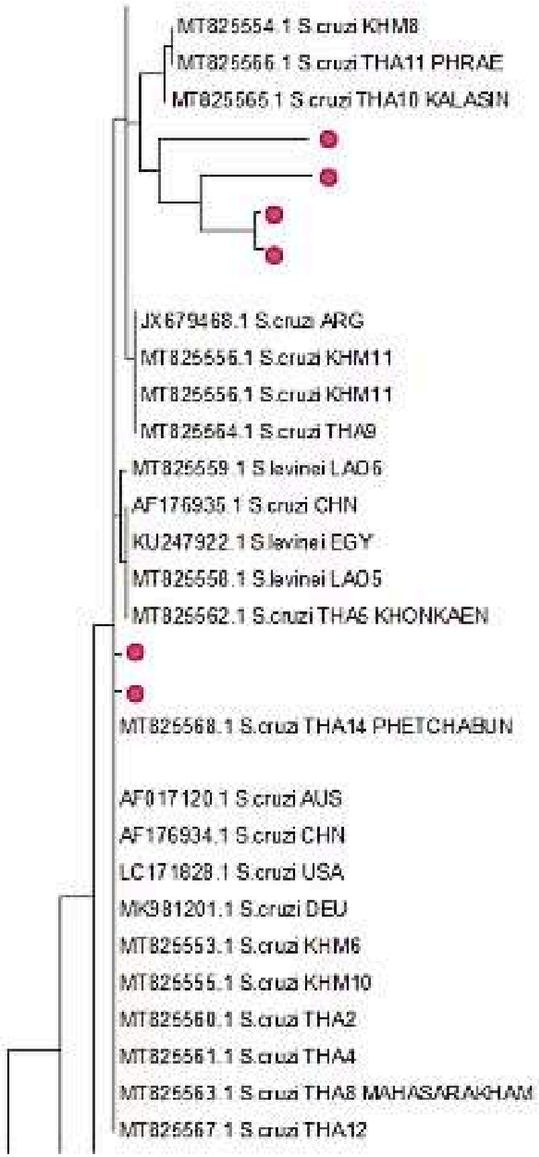
Clinicopathological and Molecular Profiles of 17 Selected Cases

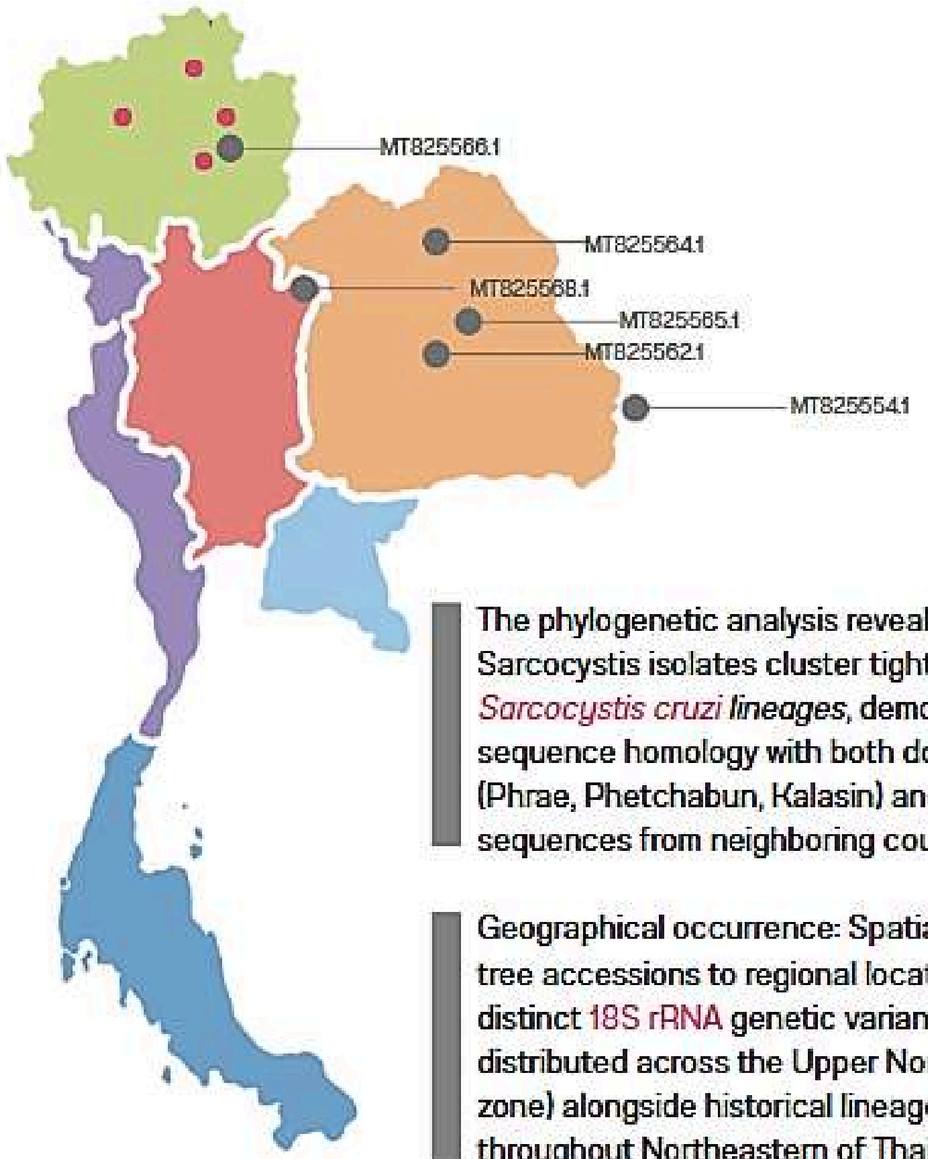
Species	Sex	Age (Y)	Sample type	Location	Clinical signs	Microscopic findings		PCR results	
						Heart	Other organs	*S. cruzi*[a]	*S. hominis*
Dairy cattle	F	8	Tissues	Mueang, Chiang Mai	Loss of appetite/Dyspnea	Mixed cysts	NSL	●	●
Dairy cattle	F	3.4	Tissues	San Pa Tong, Chiang Mai	Loss of appetite	Thin wall cysts	NSL	●	
Dairy cattle	F	8	Tissues	San Pa Tong, Chiang Mai	Loss of appetite/Depress	Thin wall cysts	NSL	●	
Cattle	F	11	Tissues	Mueang, Phrae	Emaciation	Mixed cysts	NSL	●	●
Cattle	F	11.5	Tissues	Mueang, Phrae	Emaciation/Depress	Thin wall cysts	NSL	●	
Cattle	F	9	Tissues	Mueang, Phrae	Weakness/Depress	Thin wall cysts	NSL	●	
Cattle	F	9	Tissues	Mueang, Phrae	Weakness/Depress	Thin wall cysts	NSL	●	
Cattle	F	11	Tissues	Mueang, Phrae	Weakness/Depress	Thin wall cysts	NSL	●	
Cattle	F	NA	Tissues	Mae Suai, Chiang Rai	NA	Thin wall cysts	NSL	●	
Cattle	F	4.6	Tissues	Wiang Chiang Rung, Chiang Rai	Respiratory sign	Thin wall cysts	Pulmonary edema	●	
Cattle	F	4	Tissues	Pan, Chiang Rai	Weakness/Depress/Fever	Thin wall cysts	NSL	●	
Cattle	F	10	Tissues	Mueang, Chiang Rai	NA	Mixed cysts	NSL	●	●
Cattle	F	2.4	Tissues	Phu Kamyao, Phayao	Loss of appetite/Depress/ Epistaxis	Thin wall cysts	NSL	●	
Cattle	F	2	Tissues	Phu Kamyao, Phayao	Dyspnea	Thin wall cysts*	Pleuritis and pneumonia	●	
Cattle	F	4	Tissues	Phu Kamyao, Phayao	Weakness/sternal recumbency	Thin wall cysts	NSL	●	
Cattle	F	11.1	Tissues	Mueang, Phayao	Weakness/sternal recumbency	Thin wall cysts	NSL	●	
Cattle	F	10	Tissues	Dok Khamtai, Phayao	Weakness	Mixed cysts	NSL	●	●

*; Tissue cysts with cellular reaction, NSL; No significant lesion, a; PCR was performed by targeting 18S, ●; PCR positive

Results

Phylogenetic analysis



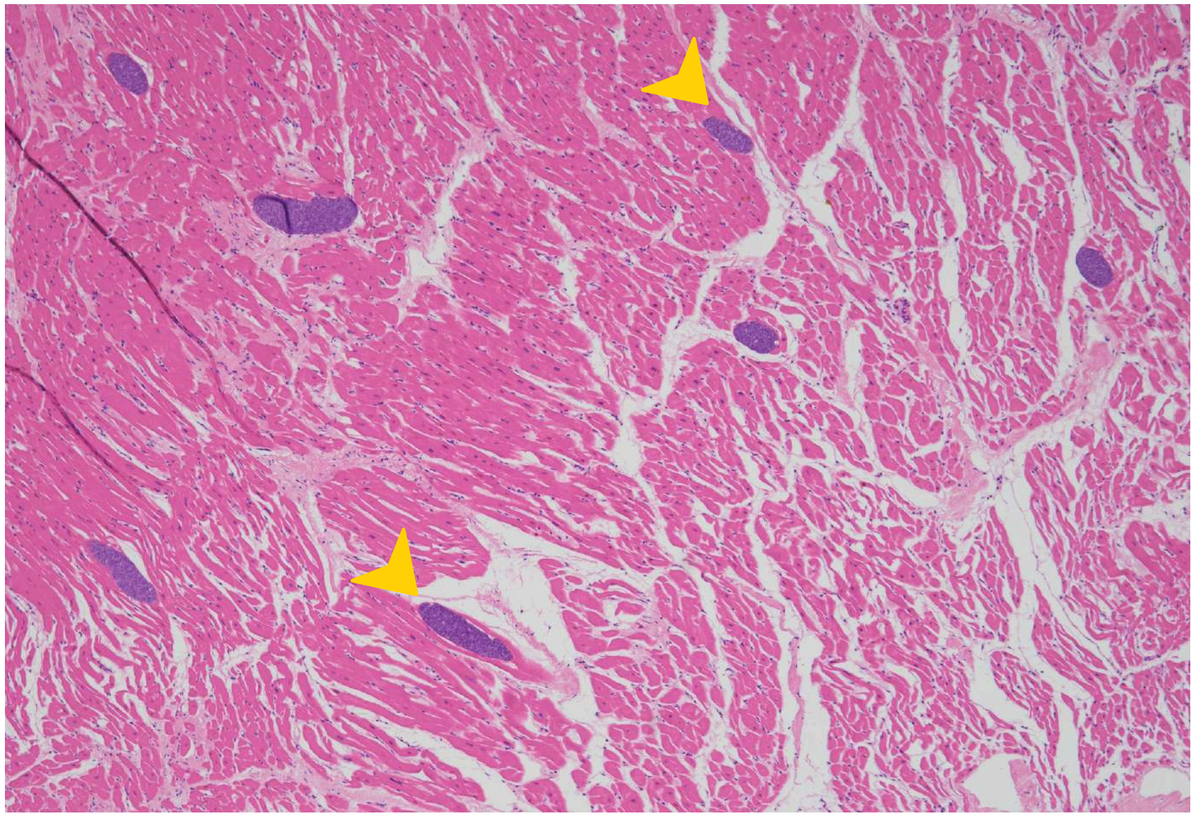


The phylogenetic analysis reveals that the local Sarcocystis isolates cluster tightly within the *Sarcocystis cruzi lineages*, demonstrating high sequence homology with both domestic Thai strains (Phrae, Phetchabun, Kalasin) and regional reference sequences from neighboring countries.

Geographical occurrence: Spatial mapping correlating tree accessions to regional locations indicates that distinct 18S rRNA genetic variants are broadly distributed across the Upper Northern region (green zone) alongside historical lineages identified throughout Northeastern of Thailand.

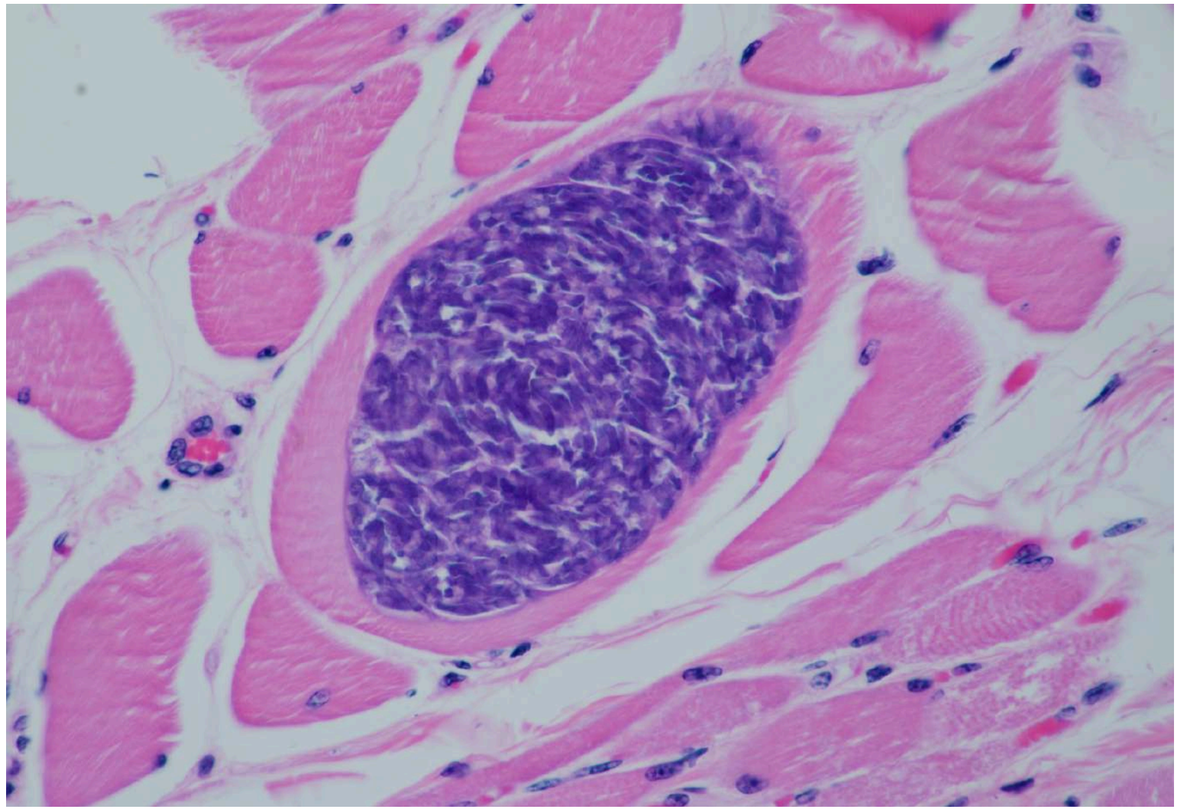
Results

Histopathological examination of heat tissue and cyst comparison



.Bovine heart tissue, HE staining

Basophilic, spindle-to-oval intramyocytic cysts (varied in shape) embedded within cardiomyocytes (arrow heads)



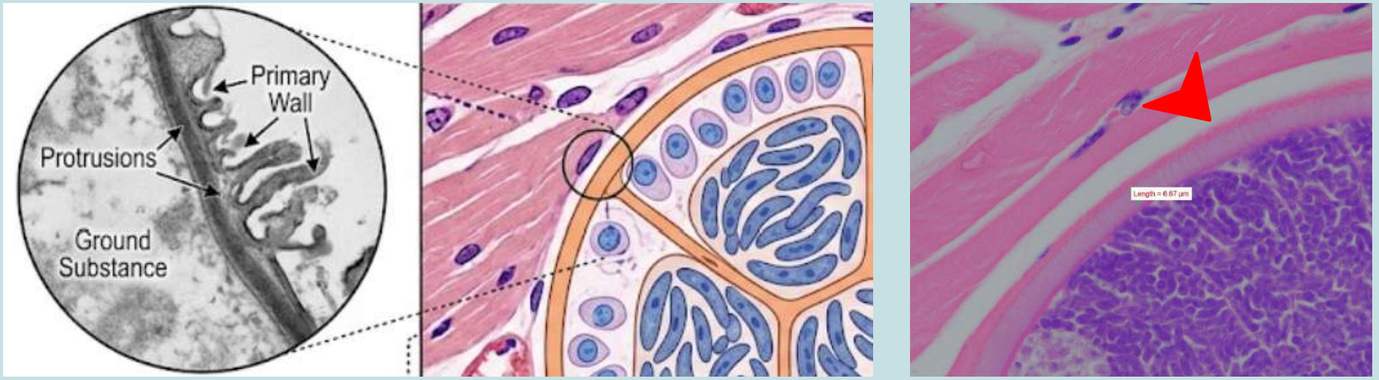
.Bovine heart tissue, HE staining

High-power structural morphology, pointing out the outer cyst wall encapsulating tightly packed crescentic bradyzoites.

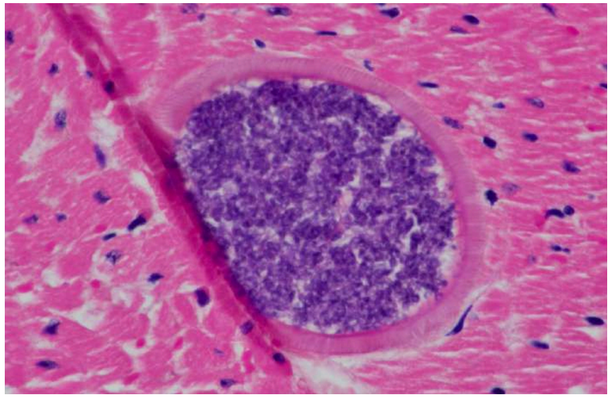
Results

Histopathological examination and cyst comparison

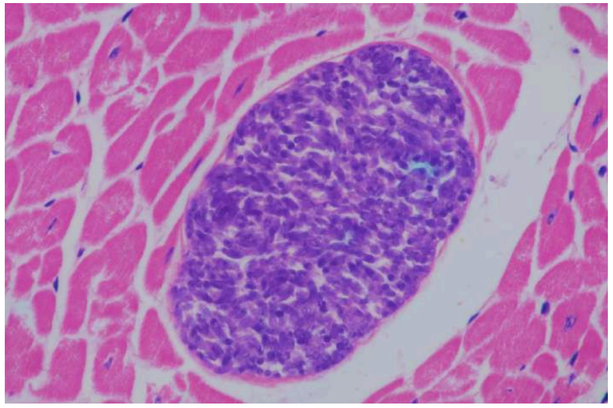
Basic structure of cyst



Thick wall cyst



Thin wall cyst



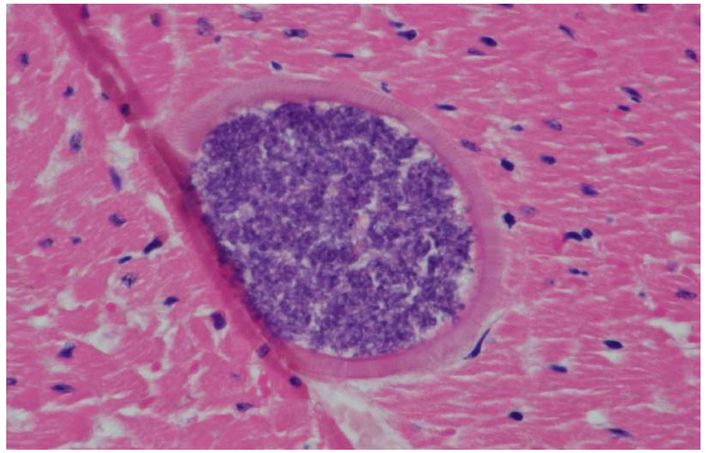
Cyst shape	Cyst wall thickness		Cyst diameter	
	Thin-walled (n=30)	Thick-walled (n=16)	Thin-walled (n=30)	Thick-walled (n=16)
Oval-Spherical Elliptical-Elongated Fusiform/Spindle-Shaped Irregular/Lobulated	6.45 ± 1.11 µm	1.99 ± 0.40 µm	55.97 ± 6.31 µm	56.26 ± 5.70 µm
	$p < 0.001$		$p = 0.8771$	

Data expressed as Mean ± SD. Statistical comparison by Independent Samples t-test

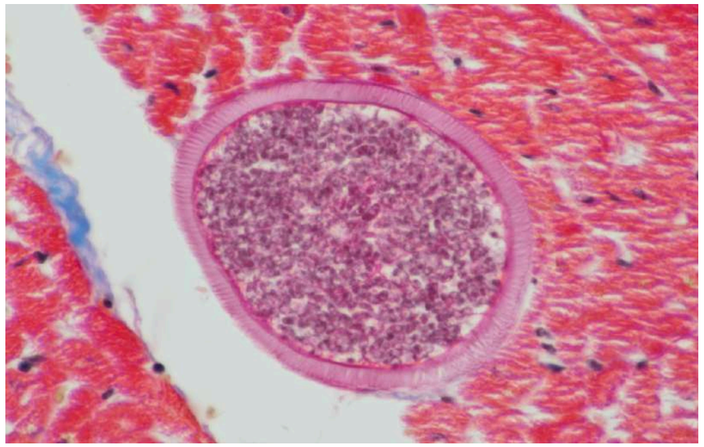
- Detected Sarcocystis tissue cysts were categorically divided into two distinct groups based on wall structure: thin-walled cysts and thick-walled cysts.
- A highly significant difference was observed between 2 groups, with thin-walled cysts and thick-walled cysts.
- In contrast to wall thickness, the overall cyst diameters were statistically comparable between the groups.

Results

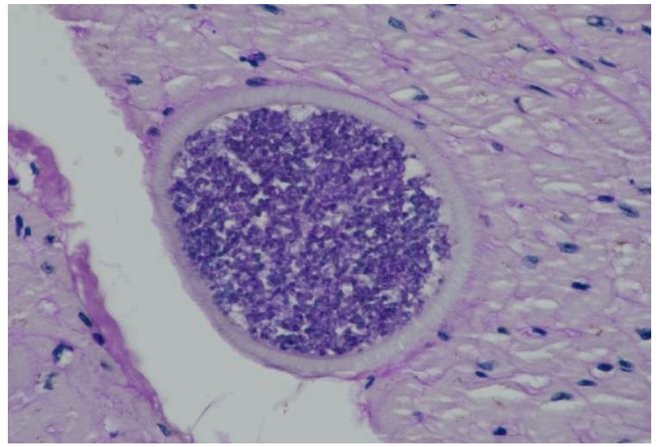
Histopathological examination and cyst comparison



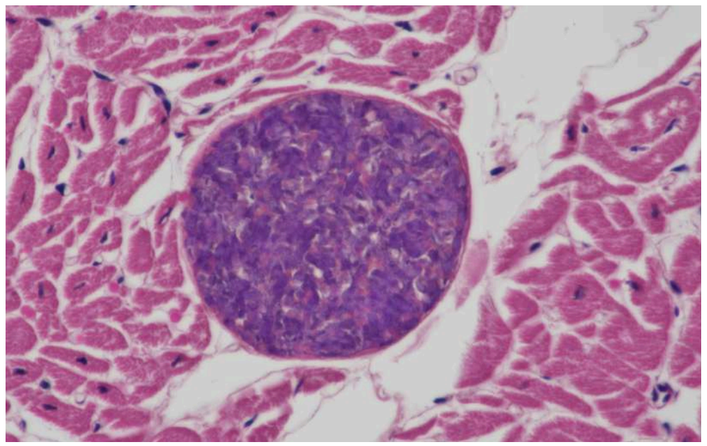
Thick wall cyst, HE staining



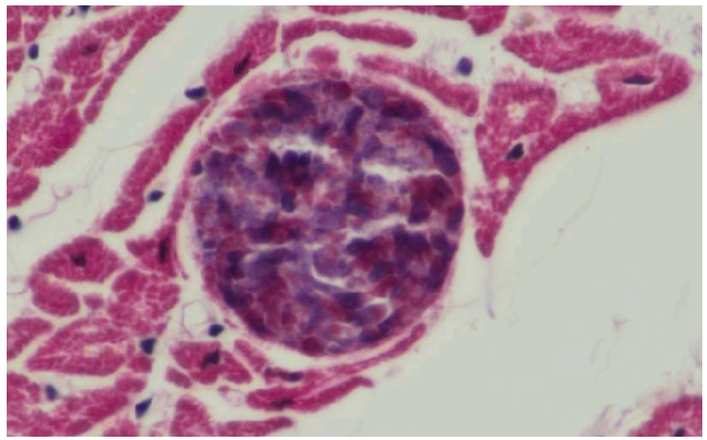
Thick wall cyst, Masson's trichrome staining



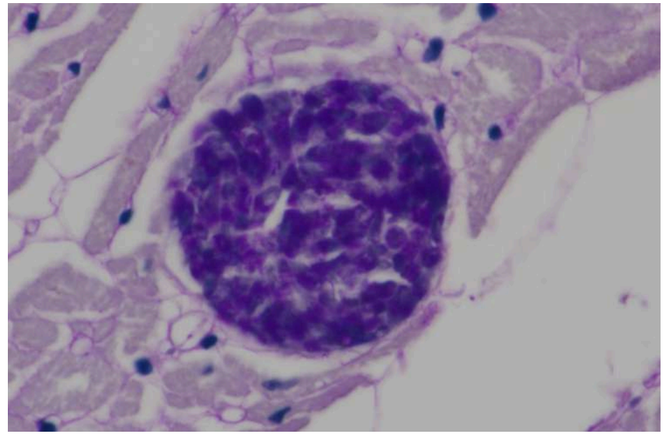
Thick wall cyst, PAS staining



Thin wall cyst, HE staining



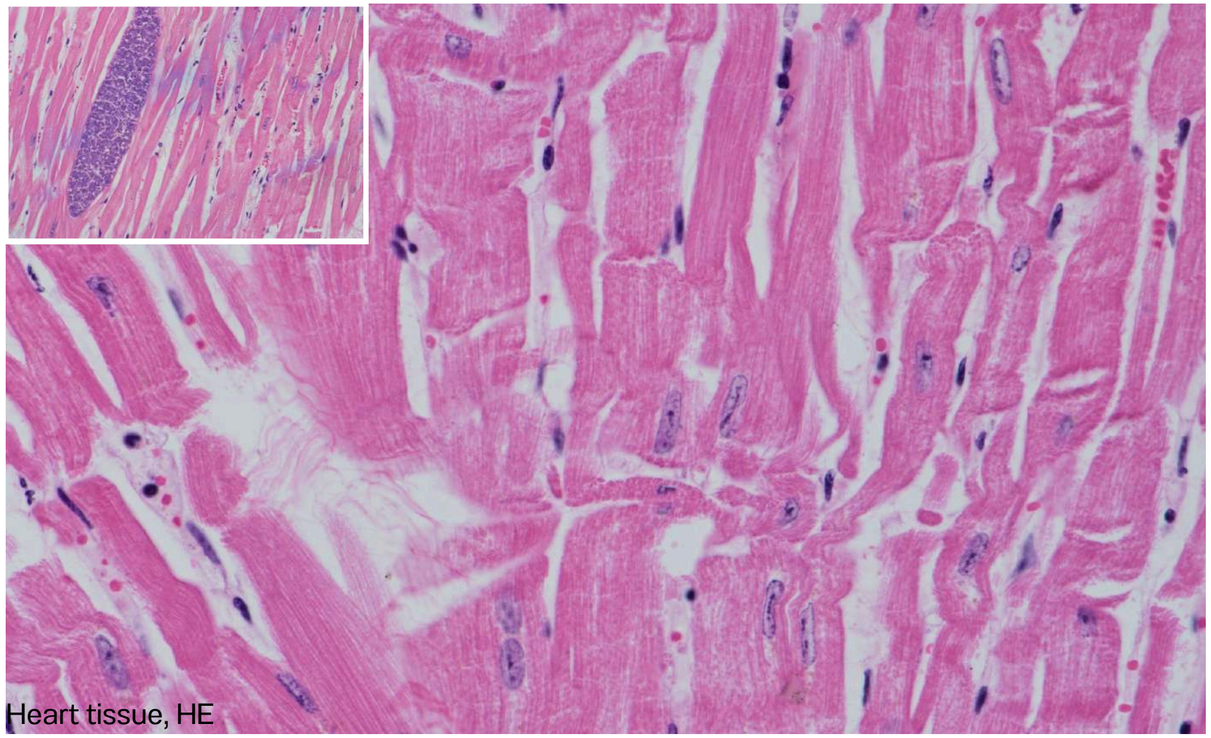
Thin wall cyst, Masson's trichrome staining



Thin wall cyst, PAS staining

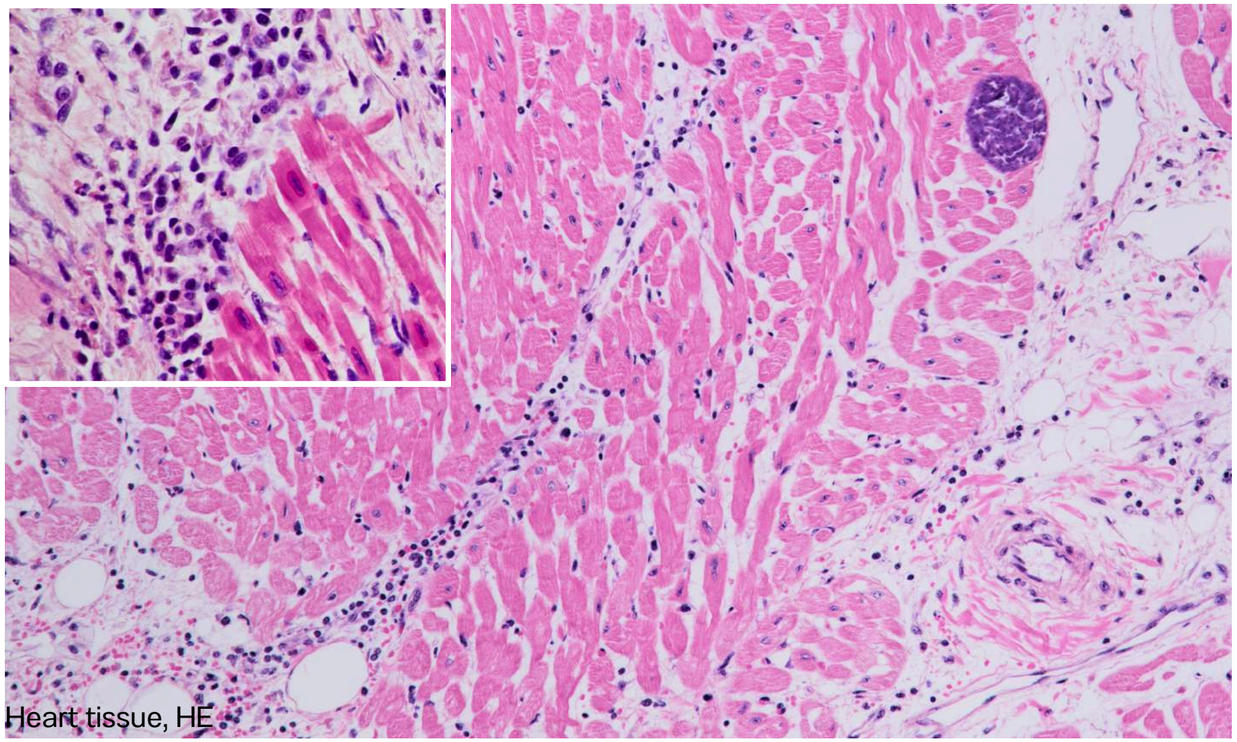
Results

Other histopathological findings



Heart tissue, HE

Myocardial interstitial edema and degeneration: separation of adjacent cardiac muscle bundles and individual myofibers due to clear space accumulation, indicating interstitial edema. Cardiomyocytes exhibit swelling, loss of cross-striations, and caterpillar cells, reflecting cellular injury

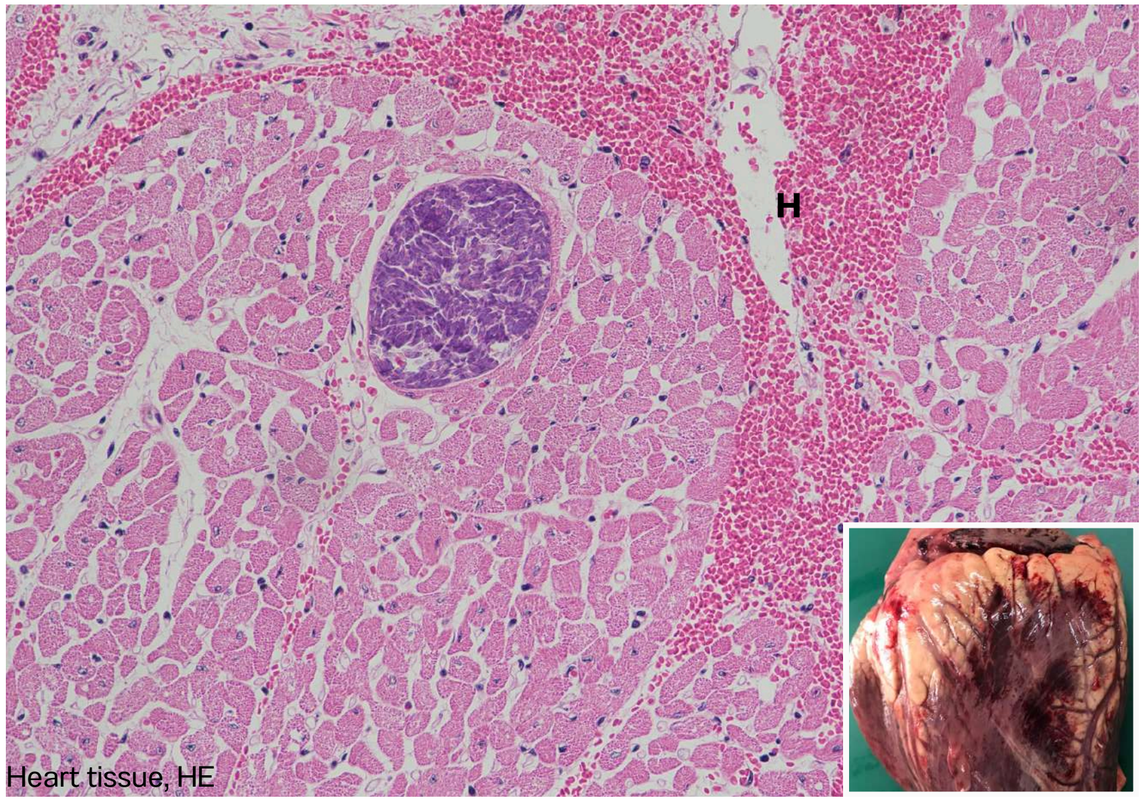


Heart tissue, HE

Interstitial myocarditis and fibrovascular proliferation: a distinct, focal-to-linear infiltration of mononuclear inflammatory cells (predominantly lymphocytes and plasma cells) tracking along the interstitial spaces, suggesting (lymphoplasmacytic) interstitial myocarditis.

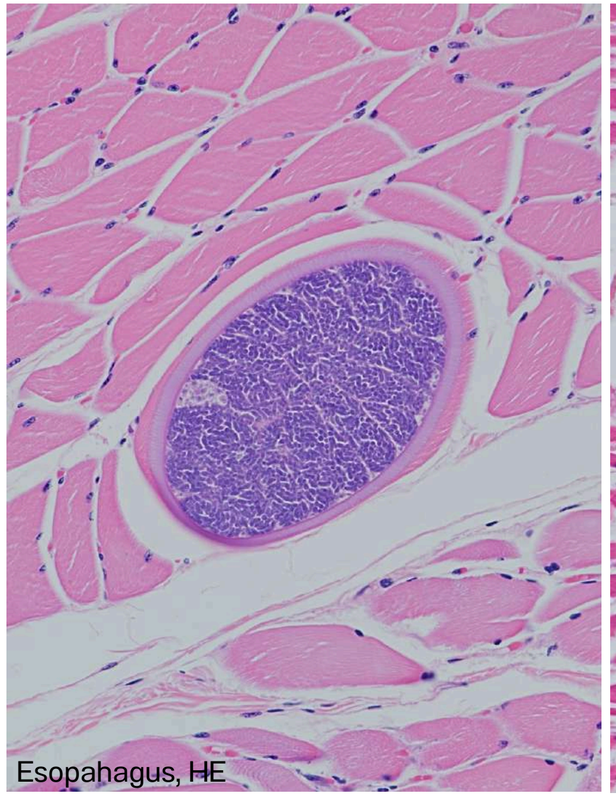
Results

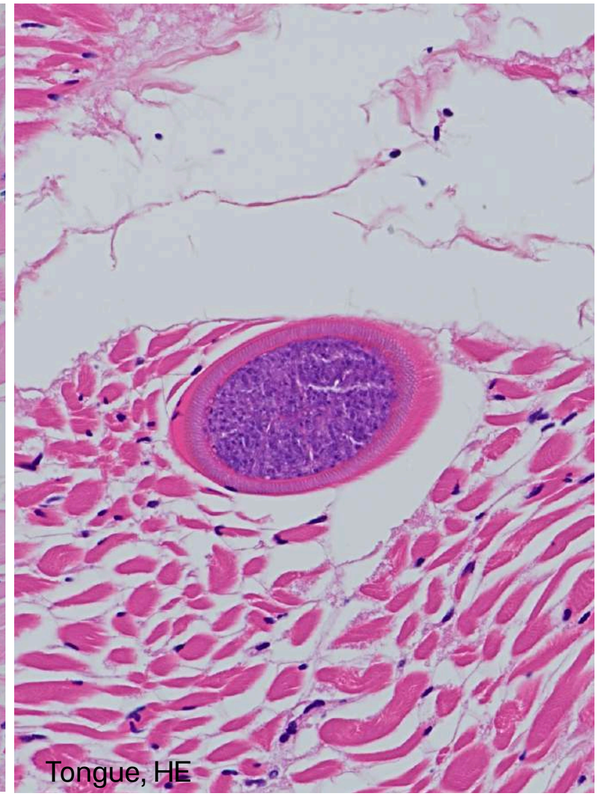
Histopathological findings



Heart tissue, HE

Severe interstitial hemorrhage: the adjacent myocardium reveals a large, prominent zone of interstitial hemorrhage (H), massive collections of extravasated red blood cells heavily infiltrate and separate the myocardial muscle bundles surrounding a tissue cyst.

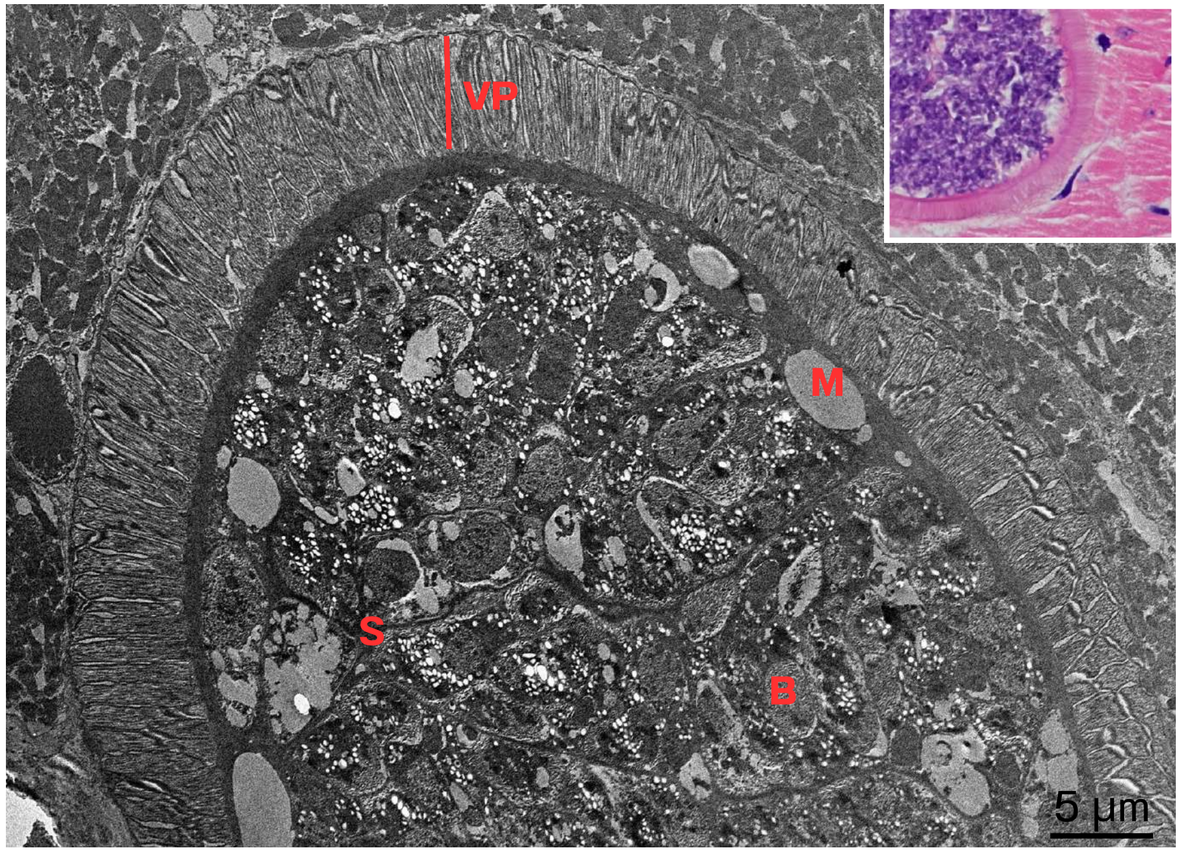

Esopahagus, HE


Tongue, HE

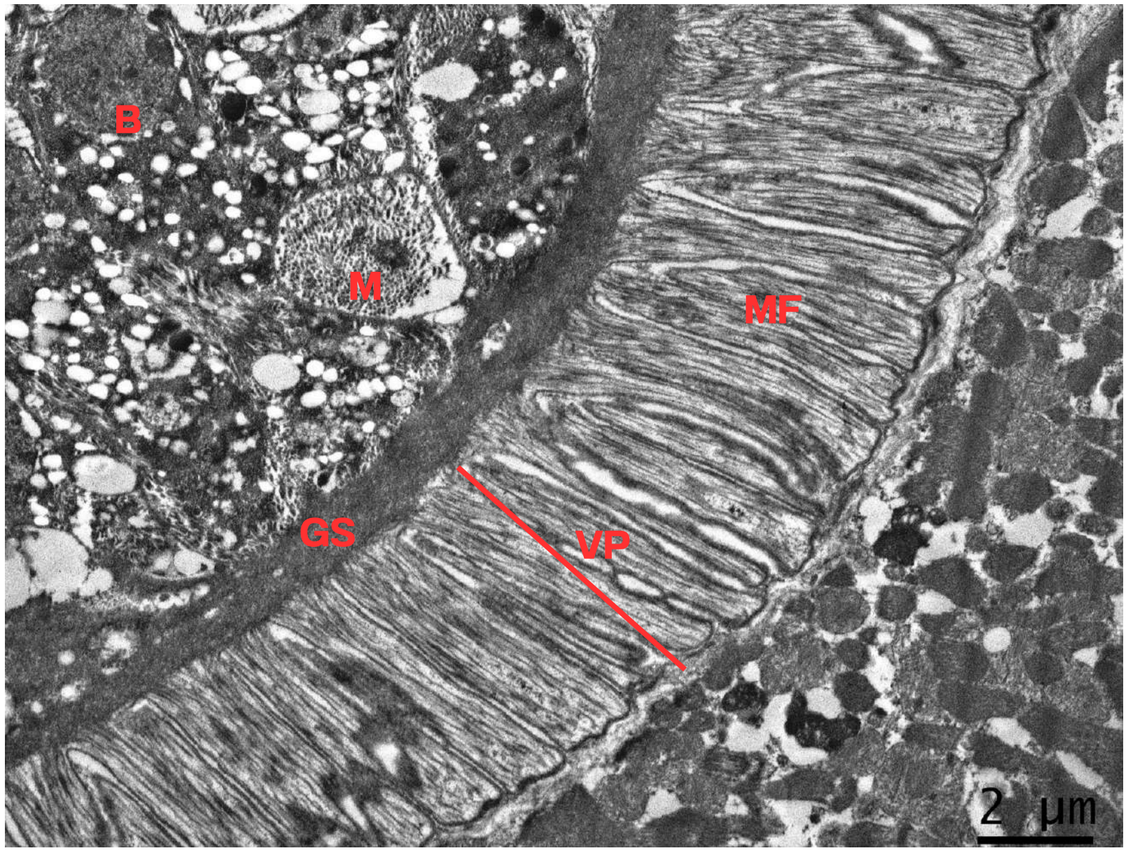
Intramyocytic sarcocysts: oval-to-elongated tissue cysts are observed developing within the cytoplasm of skeletal myofibers in the esophagus (left) and tongue (right). The cysts revealed thick, well-defined wall and are densely packed with basophilic bradyzoites running parallel to the unaltered host myofibers.

Results

Ultrastructural examination of thick-walled cyst (Transmission Electron Microscope; TEM)



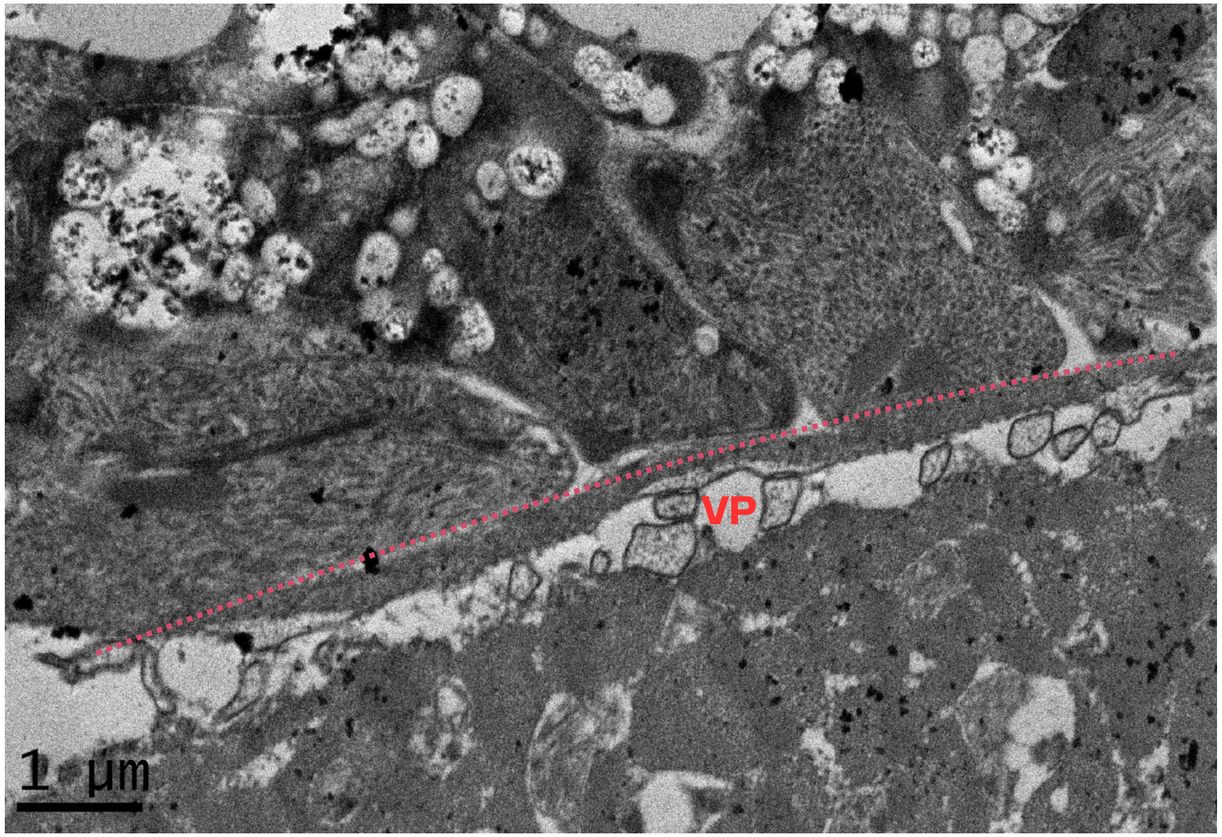
Low magnification of a cross section, thick-walled sarcocyst. The cyst is densely packed with bradyzoites in the compartments separated by septa (S) and several large, pale peripheral metrocytes (M), enclosed by a prominent, radially striated primary wall 4-5 µm in thickness.



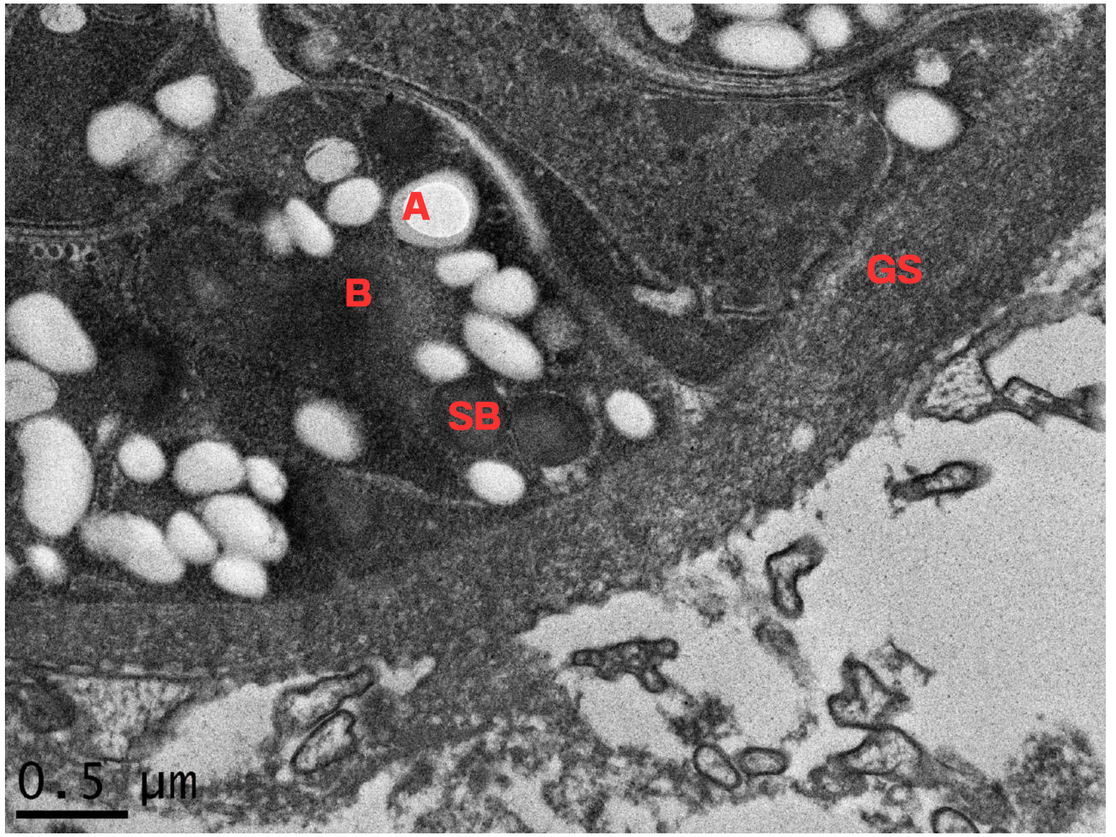
High magnification of a cross section of a thick-walled sarcocyst. Cylindrical, finger-like villar protrusions (VP) extend into the host cell, and internal microfilaments (MF) are observed running parallel to the long axis of the protrusion **a characteristic hallmark of *S. hominis***. Metrocytes (M) and bradyzoites (B) are also present within the cyst matrix.

Results

Ultrastructural examination of thin-walled cyst (Transmission Electron Microscope; TEM)



High-magnification view of a thin-walled sarcocyst, demonstrating the characteristic wall structure of *S. cruzi*. The primary cyst wall is extremely thin (dotted line, less than 1 µm), featuring short, hair-like or flattened villar protrusions (VP) distinctly lack internal microfilaments.



An ultra-high-magnification view showing the delicate, thin boundary of the cyst wall. A bradyzoite (B) is identifiable by its densely arranged internal organelles, including amylopectin granules and dark apical secretory bodies. The extremely thin primary wall lacks microfilaments and is arranged on top of the dense, dark layer of the cyst wall.

Key kindings

- **Ongoing regional circulation:** PCR screening of the cardiac tissue samples confirms the successful detection of *S. cruzi* as a primary agent of sarcocystosis in cattle within our local area. Genetic analysis shows that these local isolates are closely related to both Thai isolates and those found in neighboring countries, indicating a stable and ongoing transmission cycle across the region.

- **Histopathological differentiation & Co-infection**: By integrating histopathology with molecular analysis, we successfully identified mixed-species co-infections within the same cardiac tissues. Microscopic evaluation of the myocardium provided clear structural proof of these dual infections, allowing us to differentiate between concurrent thin-walled cysts (*S. cruzi*) and thick-walled cysts (*S. hominis*-like).

- **Ultrastructural confirmation by TEM**: To definitively confirm the differences between the two cyst types, transmission electron microscopy (TEM) was utilized. Under high-magnification ultrastructural view, the thick-walled variants exhibited a classic radiated profile featuring **perpendicular, cylindrical villar protrusions packed with parallel microfilaments**, providing definitive confirmation of *S. hominis*.

- **Public health implications**: Our findings raise significant public health concerns by confirming that the zoonotic parasite *S. hominis* is actively present in local beef. Because this species relies on humans as its definitive host, its continued presence in the livestock supply chain highlights a critical zoonotic risk for consumers in our region.

Conclusion and discussion

Evidence in our area

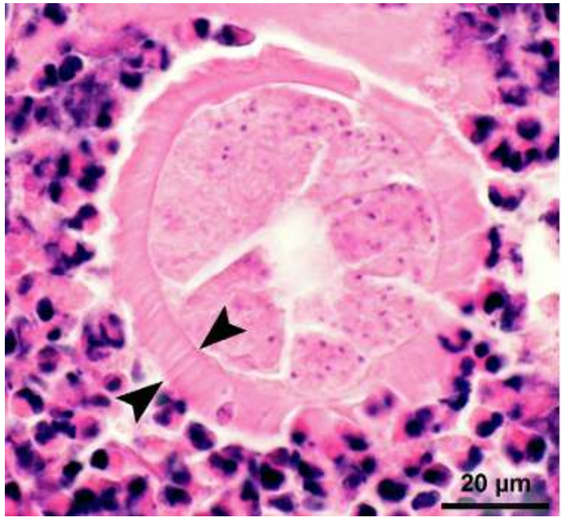
- Studies from 2007 in Thai livestock products, in one province in upper northern, revealed a 100% infection rate of microscopic Sarcocystis cysts in market meat samples. Our evidence of Sarcocystis demonstrates that this protozoan continues to actively circulate in our area. This highlights a long-standing cycle in local animal populations that requires species identification and ongoing surveillance.

No visible gross lesions or macroscopic cysts

- There are no visible gross lesions or macroscopic cysts detectable by the naked eye during routine carcass inspection. (Dubey et al., 2016)

Bovine Eosinophilic Myositis (BEM)

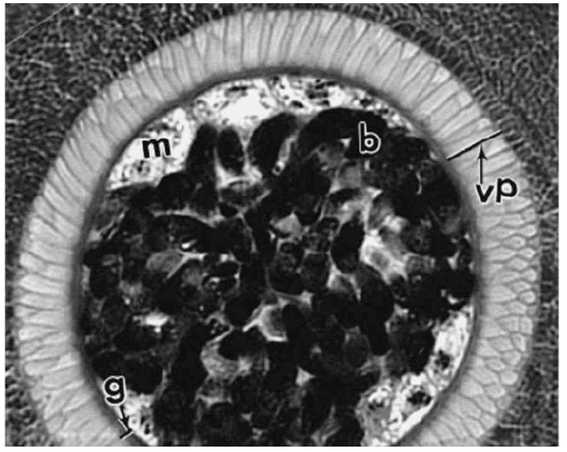
- Cell reaction lesions are frequently absent; severe cyst degeneration or rupture–particularly involving *S. hominis* can still trigger BEM in other striated muscles like the diaphragm, tongue, and esophagus. Because these reactions can manifest in other organs while sparing the heart, further study is required to fully clarify the tissue distribution and pathogenesis of *S. hominis*.



Dubey and Rosenthal, 2023

Histological evaluation of cyst wall thickness remains a highly reliable

- Light microscopy plays as an effective initial screening tool, categorizing thin (less than 1 µm) and thick (greater than 5 µm) cyst walls to differentiate *S. cruzi* from zoonotic species like *S. hominis* (Dubey et al., 2016). However, definitive species confirmation relies on transmission electron microscopy (TEM) to resolve the primary cyst wall's structure. By integrating both techniques, we successfully cross-verified mixed-species infections. Our TEM findings definitively confirmed *S. cruzi* by its thin wall and flattened villar protrusions lacking internal microfilaments (Jeon et al., 2018), while confirming co-infecting *S. hominis*-like cysts by their thick wall and cylindrical protrusions packed with parallel longitudinal microfilaments (Pena et al., 2001).



Wouda et al., 2006

Discussion

"Thick-Wall" public health concerns

- Histopathological identification of a thick-walled, "*S. hominis-like*" cyst immediately signals a foodborne protozoan threat, further molecular species differentiation is mandatory because other non-zoonotic thick-walled species (like *S. bovifelis* and *S. hirsuta*) share the same tissue layer and can cause confusion.

Increase awareness in Northern Thai

- Human exposure is highly elevated in the upper northern region of Thailand due to the popular cultural tradition of consuming raw or undercooked meat dishes like larb dip and lu. As a major public health concern because multiple species can cause intestinal sarcocystosis in humans (triggering acute nausea, vomiting, and severe diarrhea)

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Thank you for listening

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