IJVAR

International Journal of Veterinary and Animal Research

Journal homepage: https://ijvar.org/index.php/ijvar

Pathology of Concurrent Infection of Metastrongylus apri with *Trichuris suis* in a Yorkshire Pig

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ARTICLE INFO

Received: 23/04/2025
Accepted: 23/07/2025

DOI: 10.5281/zenodo.17068531

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Keywords

Coinfection
Colitis
Parasitic diseases
Pneumonia
Swine

Cite this article as: *Sewoyo P.S., Permana R., Putra I.P.C., Suandhika P. 2025. Pathology of concurrent infection of metastrongylus apri with Trichuris suis in a yorkshire pig. International Journal of Veterinary and Animal Research, 8(2): 49-53. DOI: 10.5281/zenodo.17068531.*

ABSTRACT

A two-month-old Yorkshire pig from Undisan Village, Bangli Regency, Bali, Indonesia was found dead, with a history of coughing, decreased appetite, and bloody diarrhea. Fecal examination revealed the presence of *Metastrongylus* spp. and *Trichuris suis* eggs. Gross pathology findings included verminous pneumonia, hemorrhagic gastritis, and hemorrhagic typhlitis. Worms were observed in the lungs, as well as on the mucosal surface of the large intestine and cecum. The worms inside the lungs was identified as *M. apri*, while those in the colon and cecum were *T. suis*. Histopathological examination revealed mild tracheitis with epithelial deciliation and goblet cell proliferation, emphysematous bronchiolitis, hemorrhagic gastritis, and hemorrhagic typhlitis. Based on these findings, it can be concluded that the pig was diagnosed with Metastrongylosis and Trichuriosis.

INTRODUCTION

The pigs are susceptible to infections by various parasites, including helminths. Parasitic infections such as helminthiasis can significantly impact the health and productivity of pigs, leading to economic losses in the pig farming industry (Panayatova-Pencheva et al., 2019; Kouam and Ngueguim, 2022). These effects include reduced growth rates, weight loss, and increased mortality. Additionally, the cost of anthelmintic treatments for worm control is considered high by many farmers (Nwafor et al., 2019).

Metastrongylosis is a lungworm infection caused by nematodes of the genus *Metastrongylus*, which affect both wild boars and domestic pigs through an intermediate host, earthworms. Pigs become infected when ingesting earthworms containing third-stage larvae of *Metastrongylus* spp. (Wallgren and Pettersson, 2022). Several species of *Metastrongylus* are present (Gassó et al., 2014). Among these species, *M. apri* is the most common infecting pigs (Helke et al., 2015). The lung worms can cause growth disorders in infected pigs. Clinically, affected pigs exhibit coughing due to bronchitis (Frontera et al., 2005; López and Martinson, 2017). Moreover, the significant importance of this lungworm is its zoonotic potential (Calvopina et al., 2016; Ghahvei et al., 2024). Human infections have been reported in Europe and America, with clinical signs of coughing with bloody sputum and progressive dyspnea (Calvopina et al., 2016).

A study by Oliveira et al. (2023), reported a 17.2% (10/58) prevalence of *M. apri* in necropsied wild boars in Brazil. In Uganda, *Metastrongylus* spp. were detected in 7.6% (95% confidence interval [CI]) of 932 pigs based on fecal sample analysis (Roesel et al., 2017). Similarly, a prevalence of 10.4% (95% CI: 8.1-13.2) was reported in Cameroon from a total sample of 597 pigs (Kouam and Ngueguim, 2022). The last epidemiological report on Metastrongylosis in Bali, Indonesia, was published by Dharma et al. (1988). Their study examined 104 pig lung specimens from the Denpasar Slaughterhouse and found *M. apri* infections in 21 pigs (21.19%), with larval migration lesions observed in two cases (1.92%). More recently, a study in Kupang, East Nusa Tenggara, Indonesia reported an 18% prevalence of *Metastrongylus* spp. based on coprological examination of 100 samples (Chrismanto et al., 2023).

Trichuriosis in pigs is an infection caused by the whipworm *Trichuris suis* (Bünger et al., 2022). *T. suis* is globally distributed and can infect pigs as well as several primate species, including humans. The parasite is transmitted via ingestion of soil contaminated with infective eggs. Once ingested, the larvae hatch in the ileum, cecum, and colon. High infection doses of *T. suis* can lead to clinical signs such as anorexia, anemia, and diarrhea, which contribute to weight loss. In some cases, blood or mucus may be present in the feces (Pittman et al., 2010).

Several studies have reported the prevalence of *T. suis* infection in pigs. In Chongqing, China, the prevalence was 10.13% among 2971 pigs (Lai et al., 2011). A similar rate of 20% was reported in Myanmar among 500 pigs (Bawm et al., 2024). In Bali, Indonesia, the prevalence was notably higher, reaching 71.7% among 117 pigs (Pinatih et al., 2024). This case report describes the pathological features of a pig co-infected with Metastrongylosis and Trichuriosis.

MATERIALS AND METHODS

Case history

A two-month-old Yorkshire pig from Undisan Village, Bangli Regency, Bali, Indonesia (8°26'20.3"S 115°23'43.4"E) experienced decreased appetite, lethargy, coughing, and bloody diarrhea starting on January 1, 2022, according information from the owner. The pig was housed in cages with cement floors. Previously, there was a history of pig deaths with similar symptoms. The pig died on January 8, 2022, due to lack of treatment. The carcass was subsequently necropsied at the Laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, Udayana University, Indonesia.

Pathology examination

Prior to necropsy, a fully comprehensive external examination of the carcass was conducted. The necropsy procedure was then performed, with pathological evaluation of the organs conducted both *in situ* and *ex situ*. Gross pathological changes were documented, and affected organs were sampled by excising tissue sections measuring 1×1×1 cm. The preparation of histopathology slides followed the standard protocol of the Laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, Udayana University. After sectioning, tissue samples were immediately fixed in 10% neutral buffered formalin for 24 hours. Following fixation, the samples were rinsed under running water and placed into labeled tissue cassettes. The cassettes were then loaded into an automated tissue processor for dehydration (using graded alcohol), clearing (with toluene), and paraffin infiltration. After processing, the tissues were embedded in paraffin to form blocks and then sectioned at a thickness of 5 µm. Then the tissues were processed and stained using Harris Hematoxylin and Eosin (H&E) staining, following the protocol described by Sewoyo et al., (2022). The histological slide was then examined under a microscope at 100× and 400× magnification.

Parasitology examination

Worm samples in the lungs were collected by incising the part that had undergone gross pathological changes, while worm samples in the cecum were collected directly from the mucosa. Worms were identified morphologically directly under a microscope at 40× magnification without preservation or staining. The species of worms *Metastrongylus* spp. were classified based on the morphological identification key of Gassó et al., (2014). *T. suis* samples were identified morphologically based on Otranto and Wall (2024). Fecal samples were also collected for the purpose of examining the morphology of worm eggs using native, sedimentation, and floating methods. Floatation examination uses a saturated salt floatation agent (Zajac et al., 2021).

RESULTS

The identification of worms was based on the morphological characteristics of adult worms, their eggs, and their predilection sites (Figure 1). *Metastrongylus* spp. were retrieved from the caudal lobes of both the left and right lungs, while *T. suis* was found on the mucosal surface of the cecum.

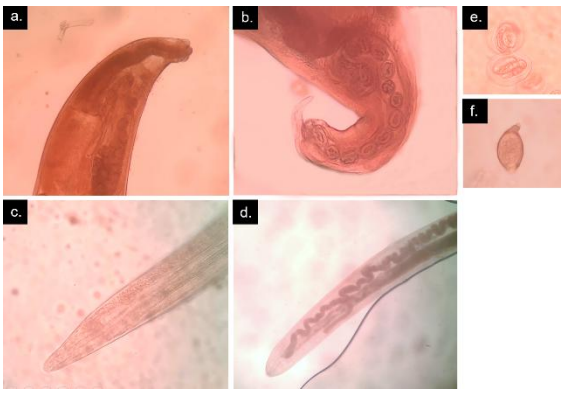

Figure 1. Morphological characteristics of *Metastrongylus apri* (a, b, e) and *Trichuris suis* (c, d, f). (a) Anterior end of a female *M. apri*. (b) Posterior end of a female *M, apri*. (c) Posterior end of a female *T. suis*. (d) Posterior end of a female *T. suis*. (e) *M. apri* eggs identified using the sedimentation method. (f) *T. suis* eggs detected using the flotation method with a saturated salt solution. (100× and 400× magnification)

The *Metastrongylus* spp. identified as *M. apri* based on morphological examination. The *M. apri* specimens collected were adult female worms (Figure 1a,b). Morphologically, the posterior end (Figure 1b) lacked prevulvar cuticular dilatation, with the vulva positioned slightly anterior to the anus and posterodorsal to the prevulvar swelling. The anterior end displayed characteristic trilobed labia (Figure 1a). *M. apri* eggs,

identified through sedimentation examination, were ellipsoid in shape, with a thick, rough shell and were larvated upon excretion.

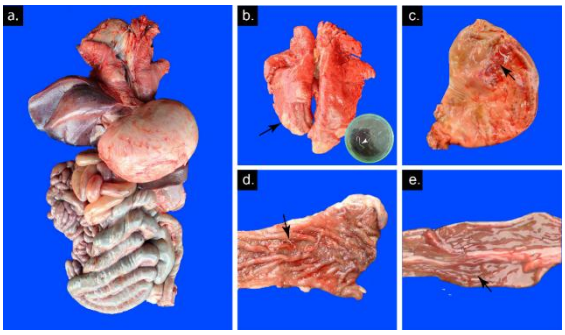

Figure 2. Gross pathology of pig internal organs. (a) Gross pathology of organs *ex situ*: The stomach and intestines appear distended, with hyperemia on the serosal surface of the small intestine and colon. (b) Lungs: The lungs were hyperemic with multifocal areas of inflammation (arrows), and worms are present (arrowheads). (c) Stomach: Hemorrhage in the gastric mucosa. (d) Cecum: Thickening of the mucosa, with hemorrhage and the presence of worms (arrows). (e) Small intestine: Hyperemic small intestinal mucosa

T. suis worms appeared as white, whip-like structures adhered to the cecal mucosa during gross examination (Figure 2d). The female worms exhibited a distinct morphology, with a slender anterior end and a thick, curved posterior end. *T. suis* eggs, detected using the flotation method, were lemon-shaped, with a thick, smooth shell and prominent transparent polar plugs at both ends. The egg contents were granular, unsegmented, and brownish in color.

Examination of the external body revealed no pathological changes. However, *ex situ* organ observation revealed abnormalities in several organs (Figure 2a). The stomach appeared distended, with the serosal surface of the small intestine and colon appearing reddish and showing prominent capillary blood vessels. The lungs were generally reddish, with a grayish lesion slightly raised at the distal ends of both lung lobes (Figure 2b). The gray lesion was suspected to be an area of inflammation. Upon incision, several worms were found in the lungs. Hemorrhage was observed on the surface of the gastric mucosa (Figure 2c). Upon opening the intestinal tract, small, hair-like worms were found in the colon and cecum (Figure 2d). The surface of the colon appeared thickened and reddish, with accompanying hemorrhage. No worms were found in the small intestine, but hyperemia was present (Figure 2e). The other organs, such as brain, esophagus, heart, pancreas, spleen, kidney, liver and bladder appeared unremarkable.

Histopathological examination of the organs revealed changes in the trachea, lungs, stomach, small and large intestine. In the upper respiratory tract, the trachea exhibited pseudostratified epithelial deciliation, goblet cell proliferation, and mild mononuclear inflammatory cell infiltration in the submucosa (Figure 3a). In the lungs, inflammatory cell infiltration was observed in the peribronchial region, accompanied by exudate in the bronchiolar lumen. Emphysema was also present (Figure 3b).

In the gastric mucosa, exudate and desquamation were noted in the columnar epithelium, while the lamina propria was infiltrated by mononuclear inflammatory cells and showed hemorrhage (Figure 3c). The small intestine appeared congested, with several blood vessels in the mucosa and submucosa were engorged with red blood cells. The lamina propria were infiltrated with mononuclear cells. The large intestine displayed mucosal erosion, along with eosinophilic and mononuclear inflammatory cell infiltration in the lamina propria. Cross-section of helminths were also present in the mucosa (Figure 3d).

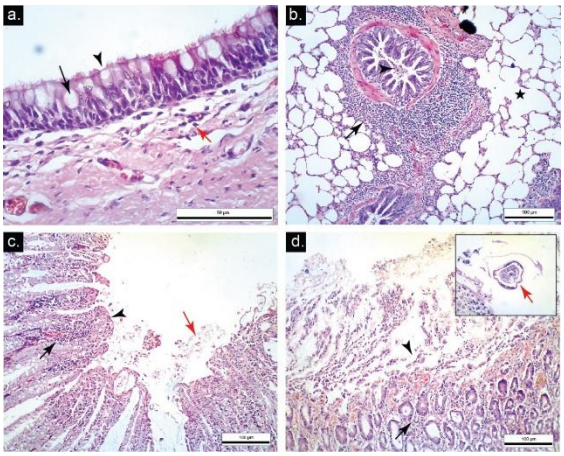

Figure 3. Histopathology of pig internal organs with Metastrongylosis and Trichuriosis. (a) Tracheitis: Deciliation (arrowhead), goblet cell proliferation (black arrow), and mild inflammatory cell infiltration in the submucosa (red arrow). (b) Emphysematous bronchiolitis: Exudate in the bronchiolar lumen (arrowhead), peribronchiolitis (black arrow), and emphysema (star). (c) Hemorrhagic gastritis: Desquamation of the mucosal epithelium (arrowhead), exudate in the lumen (red arrow), and mononuclear inflammatory cell infiltration with hemorrhage in the lamina propria (black arrow). (d) Hemorrhagic typhlitis: Erosion of the intestinal mucosa (arrowhead), eosinophilic and mononuclear inflammatory cell infiltration with hemorrhage in the lamina propria (black arrow). Cross-section of *T. suis* were observed in the mucosa (red arrow). (H&E staining, 100× and 400× magnification)

DISCUSSION AND CONCLUSION

Metastrongylus spp. is a nematode worm from the family Metastrongylidae that has a slender body shape of up to 6 cm, two lateral trilobed lips, and small dorsal bursa ray. The species are distinguished by the size and shape of the male spicules (Otranto and Wall, 2024). In addition to male morphology, worm species can be distinguished based on female morphology. Six species of *Metastrongylus* have been described, i.e., *M. apri* (also called *M. elongatus*), *M. salmi, M. pudendotectus, M. confusus, M. asymetricus*, and *M. madagascariensis* (Helke et al., 2015). The first three species are the most commonly reported worldwide and are usually found in mixed infections. Female *M. pudentodectus* and *M. asymetricus* have prevulvar cuticular dilatation, whereas *M. apri, M. confusus* and *M. salmi* do not. In distinguishing between the species *M. apri, M. confusus, and M. salmi,* the location of the vulva can be observed. In *M. apri*, the

vulva is located slightly anterior to the anus and posterodorsal to the prevulvar swelling. In *M. confusus*, it is also slightly anterior to the anus but situated within the prevulvar swelling. In *M. salmi*, the vulva is positioned at the ventral base of the prevulvar swelling (Gassó et al., 2014). Based on this identification key, the female *M. apri* worm was morphologically identified in the present case.

M. apri is a nematode parasite that infects the respiratory tract of both wild boars and domestic pigs. Epidemiological studies indicate that *M. apri* infections are more prevalent in wild boars than domestic pigs. Poglayen et al. (2016), reported that the prevalence of *M. apri* infection in wild boars reached 80.7% (46/57). Young pigs are more susceptible to infection and harbor higher parasite burdens (García-González et al., 2013; Poglayen et al., 2016). The prevalence and intensity of *M. apri* infection are closely associated with highland regions and areas with high rainfall, as these environments favor the presence of earthworms, the intermediate host of the parasite (Gassó et al., 2014).

Macroscopically, pigs infected with *M. apri* exhibit lesions predominantly in the apical lobes of the lungs, characterized by irregularly shaped grayish areas. Histopathological examination revealed lymphocytes and neutrophils infiltration within the lumina of the bronchi and bronchioles, desquamation of the bronchial and bronchiolar epithelium, emphysema, thickening of the alveolar septa, and peribronchial lymphoid hyperplasia (Panayotova-Pencheva et al., 2019). Clinically, *M. apri* infection manifests respiratory symptoms such as coughing and dyspnea, which may be exacerbated by co-infection with other parasites, such as *Ascaris suum* (Frontera et al., 2005; 2007). Coinfection has also been reported to intensify the immune response (Frontera et al., 2007). Effective control of *Metastrongylus* infection can be achieved through anthelmintic administration, particularly ivermectin and abamectin, both of which demonstrate high efficacy in reducing parasite burdens (Lopes et al., 2014).

Trichuris worms have whip-like characteristics and are also called whipworms because their thick rear end narrows into a long, threadlike front end (Bünger et al., 2022). The front end of the worm is pointed and embedded in the mucosa. The male worm has a tightly coiled tail and a single spicule in a sheath that can protrude, whereas the female is curved. *T. suis* eggs have a characteristic lemon-like shape, measuring 50-68 × 21-31 µm, with a thick, smooth shell and prominent transparent polar plugs at both ends. The species that infects the large intestines (cecum and colon) of pigs is *T. suis* (Otranto and Wall, 2024). *T. suis* is a globally distributed parasite of pigs, with prevalence rates varying by geographic location, season, and farm management practices. *T. suis* infection is of significant concern in pig farming due to its impact on growth performance and economic losses (Pittman et al., 2010; Bünger et al., 2022). Clinically, *T. suis* infection causes diarrhea, anorexia, anemia, growth retardation, dehydration, and emaciation. Brewer et al. (2019) reported that *T. suis* infection leads to typhlitis and colitis, characterized by ulceration, edema, hemorrhage, and the presence of mucoid exudate on the mucosal surface, often accompanied by adult worms. John and Saikumar (2017) described gross pathological changes in infected pigs, including cecal thickening and catarrhal inflammation. In this case, cattarhal inflammation in the cecum was not found.

Histopathological findings by John and Saikumar (2017) include multifocal necrosis of the colonic epithelium and infiltration of eosinophils and mononuclear inflammatory cells, which is in line with this study. Another study by Limarta et al. (2024) reported that severe *Trichuris suis* infection (2329 worms), accompanied by *Eimeria* sp. infection, resulted in gross pathological changes, including mucosal openings and hemorrhages in the cecum and colon, hemorrhages in the stomach and small intestine, and an uneven liver coloration with white spots. Histopathological examination further revealed necrotizing hemorrhagic enteritis, necrotizing verminous colitis, necrotizing gastritis, and necrotizing hepatitis. The gross and histopathological changes observed in that study were similar to those in our case, although to a lesser extent. In conclusion, we observed pathological changes in the trachea, lungs, and gastrointestinal tract of Yorkshire pig resulting from infection with *M. apri* and *T. suis*, confirmed by parasitological examination of the parasite. These findings confirm that the infections of both parasites still occur in Bali, Indonesia. Due to the confirmed presence of the parasite in this region and its importance regarding public health, conducting epidemiological studies is recommended to determine the current prevalence, particularly *Metastrongylus* spp.

Acknowledgement

Authors would like to express highest gratitude and appreciation to the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Udayana University, Indonesia for providing facilities for animal necropsy and histopathology examination, as well as Veterinary Parasitology Laboratory, Faculty of Veterinary Medicine, Udayana University, Indonesia for parasites examination microscopically.

Ethical Declaration

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Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: P.S., Design: P.S., R.P., Data Collection or Processing: R.P., Analysis or Interpretation: P.S., I.C., Literature Search: P.S., I.C., Writing: P.S., I.C.

Financial Support

This research received no grant from any funding agency/sector.

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