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SHORT COMMUNICATION

Gastrointestinal Parasites in Wild Boars from an Indigenous Community Settlement in Sarikei, Sarawak

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ABSTRACT

Wild boars (*Sus scrofa*) serve as significant reservoirs for diverse gastrointestinal parasites capable of infecting both wildlife and domestic pigs and may cause economic and production losses in pig and wild-boar farming.The present study focused on gastrointestinal helminths and protozoa in 23 free-ranging wild boars kept by the indigenous community in Sarikei, Sarawak.This investigation assessed the occurrence of helminth infections in wild boars from an indigenous community settlement in Sarikei, Sarawak, employing both microscopic analysis and molecular using multiplex PCR. All samples exhibited polyparasitism. Strongyles represented the highest percentage of the parasites at 87%, followed by *Eimeria* spp. 65.2%, *Entamoeba* spp. 47.8%, *Ascaris* spp. 34.8%, *Strongyloides* spp. 26.1%, and *Metastrongylus* spp. 8.7%. Multiplex PCR specifically targeting *Oesophagostomum* spp. detected the 330 bp amplicon corresponding to *Oesophagostomum quadrispinulatum* in 11 of the 23 samples (47.8%). These findings demonstrate a substantial gastrointestinal parasite burden and frequent polyparasitism in wild boars kept under free-ranging conditions and may cause economic and production losses in small-scale wild-boar husbandry. *Ascaris* spp. and *Entamoeba* spp., which have potential zoonotic risk, were detected in this study, indicating the need for further investigation in people and domestic livestock in the surrounding community. Enhanced monitoring and an integrated health approach are essential to mitigate transmission risks.

Keywords: Microscopy, PCR, formal-ether concentration technique, wild boar, gastrointestinal parasites

Introduction

Intestinal parasitic infections (IPIs) are widespread among humans and domestic animals, particularly in rural areas of Southeast Asia where sanitation, water quality, and health literacy are often inadequate (Sangaré et al., 2015; Sitotaw et al., 2020). Numerous IPIs are acquired via contaminated food, water or soil and many remain asymptomatic or present with nonspecific symptoms, contributing to underdiagnosis (Hotez et al., 2014).

In Malaysia, numerous indigenous communities reside in secluded rural regions and practice free-range livestock management, allowing wild boars and other animals to roam around houses and waterways. Limited sanitation and proper sewage systems exacerbates environmental contamination and heightens the risk of exposure to parasites (Khor and Zalilah, 2008). Wild boars (*Sus scrofa*), distributed worldwide, serve as notable reservoirs of parasites capable of infecting wild and domestic animals and, for some species, humans (Meng et al., 2009).

Gastrointestinal nematodes, including strongyle-type worms and nodular worms such as *Oesophagostomum* spp. are associated with intestinal pathology, reduced growth and lower productivity in pigs which may cause economic and production losses (Pattison et al., 1979). In Malaysian Borneo, wild boar hunting and small-scale husbandry are longstanding practices among non-Muslim indigenous communities, for whom wild boar meat represents a culturally significant and nutritionally important source of protein (Kurz et al., 2021). Wild boars are commonly encountered near indigenous settlements, where they may be temporarily kept or maintained under semi-captive free-ranging

conditions prior to consumption. This form of free-ranging management, characterised by minimal biosecurity and unrestricted contact between animals and the surrounding environment, creates conditions favourable for parasite acquisition and transmission at the wildlife–human interface (Meng et al., 2009). In rural indigenous communities where wild boars are kept under free-ranging conditions as a source of meat and potential income, heavy parasite burdens in these animals may consequently affect animal health, household-level production and food security.

Accurate identification of these parasites is essential for understanding their epidemiology. Eggs of *Oesophagostomum* spp. are morphologically indistinguishable from those of other strongylids by light microscopy, hence molecular tools are required for species-level identification (Lin et al., 2008). A validated multiplex PCR assay permits the specific detection of *O. dentatum* and *O. quadrispinulatum*, the two principal nodular worms of pigs and allows more precise characterisation of parasite fauna (Lin et al., 2008; Lin et al., 2012). Although these species are not currently recognised as human pathogens, documenting their occurrence in free-ranging wild boars kept as backyard livestock is relevant for veterinary health, small-scale production and baseline description of parasite communities in Malaysia. In addition, parasites such as *Ascaris* spp. and *Entamoeba* spp., which include species of recognised zoonotic concern, may also be present in this setting and warrant further investigation in local human and livestock populations (Hotez et al., 2014; Mohammadi et al., 2004).

Despite the likely importance of these infections, data on gastrointestinal parasitism in Malaysian wild boars remain scarce. This study aimed to characterise the occurrence and coinfection patterns of gastrointestinal helminths and protozoa in wild boars raised by an indigenous community in Sarikei, Sarawak, using microscopy and species-specific multiplex PCR for *Oesophagostomum* spp. and to provide baseline information relevant to animal health and small-scale wild-boar production in this setting.

Materials and methods

Sampling Procedure

A total of 23 stool samples from free range wild boars were collected in an indigenous people village at Sarikei, Sarawak. The collected stool samples were transferred in a sterilized stool container and were stored in 4◦C freezer upon reaching the laboratory until processed. Following collection, samples were aliquoted into 1.5 ml microcentrifuge tubes at Sarikei Hospital, Sarawak. The aliquoted samples were subsequently transported to Kuala Lumpur via domestic flight for laboratory processing and molecular analysis.

Microscopic Screening

The samples were processed using the formalin-ether concentration method (Young et al., 1979), subsequently followed by microscopic examination with Lugol's iodine stain. Parasites were identified by examining their morphological characteristics under 10X and 40X magnification (Otranto & Wall, 2024).

DNA Extraction and PCR Amplification

Genomic DNA was extracted from each stool sample using the NucleoSpin® Soil DNA extraction kit (Macherey-Nagel, Germany) according to the manufacturer's instructions, eluted in 50 µl and stored at −20°C until PCR.

Species-specific multiplex PCR was performed using the primer described by Lin et al. (2008) for the simultaneous identification of *O. dentatum* and *O. quadrispinulatum*. Two primer pairs were combined in a single reaction: OdspF (5′-GCAACAGGTACCTT AGAGCTA-3′) and OdspR2 (5′-TTGCAAAT GACATGAAACTAC-3′), which amplified a 130 bp fragment of the partial ITS-2 of *O. dentatum*, and OqspF (5′-ACTAACGTTTTACATTTGGGA-3′) and OqspR (5′-CATTCGTGTACCTTAGACGTA-3′), which amplified a 330 bp fragment encompassing partial ITS-1, the complete 5.8S rRNA gene and partial ITS-2 of *O. quadrispinulatum*.

Multiplex PCRs were set up in 25 µl reactions with 2× ExPrime Taq Master Mix (GENETBIO Inc., Daejeon, South Korea), with each reaction containing 12.5 µl of 2× Master Mix, 50 pmol of each primer (OdspF, OdspR2, OqspF and OqspR), all four primers and 1 µl of DNA template, and run under the cycling conditions of Lin et al. (2008). PCR products were separated on 2% agarose stained with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific, USA) and visualized under ultraviolet illumination; bands of 130 bp and 330 bp were interpreted as positive for *O. dentatum* and *O. quadrispinulatum* respectively.

Data Analysis

A table was tabulated to summarize the occurrence of the detected parasites. The 95% confidence intervals (CI) for infection estimates were computed using the Wilson score interval, which is appropriate for small sample sizes. Co-infection patterns were categorized into helminth–protozoan, helminth–helminth, and protozoan–protozoan combinations.

Results and discussion

A total of 23 stool samples from wild boar were examined (Table 1). Among all the samples, gastrointestinal nematodes (95.65%) recorded the highest infection rate followed by protozoan (82.61%). According to the species, strongyles was found to be the most abundant parasites in the wild boar, having an infection of 87% (20 out of 23), followed by *Eimeria* spp. 65.2% (15 out of 23), *Entamoeba* spp. 47.8% (11

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out of 23), *Ascaris* spp. 34.8% (8 out of 23), *Strongyloides* spp. 26.1% (6 out of 23) and *Metastrongylus* spp. 8.7% (2 out of 23) (Table 2).
Out of 23 stool samples examined, all samples harbored more than one parasitic nematodes and protozoan. There were no monoparasitism found in this study. Polyparasitism observed can be divided into three categories which are Helminth + Helminth (H+H), Helminth + Protozoa (H+P) and Protozoa + Protozoa (P+P) (Table 2). The polyparasitism between H+P showed the highest infection rate (78.26%) followed by H+H (17.39%) and P+P (4.35%).

Double infection comprised of 5 different pairs of the above mentioned parasites, triple infection included 6 different combinations and quadruple infection comprised of 4 different combinations of parasites. The combination of *Eimeria* spp. and strongyles demonstrated the highest infection (21.7% or 5 out of 23) followed by the co-infection between *Strongyloides* spp. with strongyles (13% or 3 out of 23). There were two combinations which demonstrated 8.7% (2 out of 23) of the studied samples that were the combination infections of *Eimeria* spp., strongyles with *Ascaris* spp. and co-infection between *Eimeria* spp., strongyles, *Ascaris* spp. *with Entamoeba* spp. All the other infection of combinations infection each exhibited 4.4% (1 out of 23) from the entire studied sample.

Species-specific multiplex PCR targeting *O. dentatum* (130 bp) and *O. quadrispinulatum* (330 bp) detected only the 330 bp amplicon, corresponding to *O. quadrispinulatum*, in 11 of the 23 samples (47.8%). No amplification at 130 bp was observed.
This study addressed the limited information on gastrointestinal parasites in Malaysian wild boars and showed a high parasitic burden dominated by strongyle-type nematodes, with four nematode and two protozoan species identified. The significant occurrence of parasitic infection are likely related to free-ranging management system, absence of anthelmintic treatment and inadequate hygiene practices among the indigenous community settlement, which favour environmental contamination and continual exposure of animals to infective stages (Khor and Zalilah, 2008; Hajare et al., 2022).

The overall helminth infection (95.65%) in this study exceeded reports from Russia (25%), Iran (88%) and Thailand (82.41%) (Mansouri et al., 2016; Tabakaeva et al., 2024; Thanasuwan et al., 2024). Conversely, the protozoan infection (82.61%) was also higher than values from Western Iran (67%) and Russia (33.3%) (Mohammadi et al., 2004; Belov et al., 2022). Co-infections were common, with helminth-protozoan combinations most frequent (78.26%), followed by helminth-helminth (17.39%) and protozoan-protozoan (4.35%) co-infections. Such polyparasitism can exacerbate subclinical gastrointestinal disease and may further compromise growth and productivity in wild boars kept for meat (Pattison et al., 1979; Hale et al., 1981).

Species-specific multiplex PCR demonstrated that nearly half of the wild boars were infected with *O. quadrispinulatum*, whereas *O. dentatum* was not detected. *Oesophagostomum quadrispinulatum* is a recognised nodular worm of pigs associated with intestinal lesions and reduced performance in pigs, suggesting that it may also contribute to gastrointestinal morbidity in these free-ranging wild boars (Lin et al., 2012; Pattison et al., 1979). However, not all samples with strongyle-type eggs were positive in the *Oesophagostomum* multiplex PCR, which is expected because the assay targets only *O. quadrispinulatum* and *O. dentatum*, while other strongyle-positive samples may therefore be infected with different strongyle nematodes of wild boars. The strongyle-type infections in PCR-negative samples therefore likely include a mixture of other strongyle nematodes and broader molecular methods would be needed to identify all strongyle species present in these wild boars.

Potential zoonotic parasites were also detected, notably *Ascaris* spp. and *Entamoeba* spp., which include species of zoonotic concern that may be transmitted to humans (Hotez et al., 2014; Mohammadi et al., 2004). Although this study did not assess infections in people or domestic livestock, their presence in wild boar faeces indicates a need for further investigation in the surrounding community and in co-habiting animals. Overall, these findings provide valuable preliminary information on parasite diversity and co-infection patterns in Malaysian wild boars and support the need for expanded sampling, molecular characterisation and integrated parasite control strategies in similar rural settings.

Table 1. Overall infection of gastrointestinal parasites in wild boar

Parasitic infection	**Microscopy**		
	N	**%**	**95% CI**
Helminth	**22**	**95.65**	**87.32-100**
Strongyles	20	86.97	73.71-100
Ascaris spp.	8	34.78	15.61-53.95
Strongyloides spp.	6	26.09	8.59-43.59
Metastrongylus spp.	2	8.70	0.00-20.59
Oesophagostomum quadrispinulatum (PCR)*	11	47.83	27.47-68.19
Protozoa	**19**	**82.61**	**67.12-98.10**
Eimeria spp.	15	65.22	45.75-84.69
Entamoeba spp.	11	47.83	27.47-68.19
Overall infection	**23**	**100**	**-**

Table 2. Types of gastrointestinal parasites in wild boar with polyparasitism

Type of Parasitsm	n	%	95% CI
Helminth+Helminth	4	17.39	6.75-36.7
Double Infection	4	17.39	6.75-36.7
Strongyles + *Metastrongylus* spp.	1	4.35	0.67-20.50
Strongyles +*Strongyloides spp.*	3	13.04	4.52-32.08
Helminth + Protozoa	18	78.26	57.82-90.17
Double infection	6	26.09	12.49-46.38
Eimeria spp. + *Strongyle*s	5	21.74	9.83-42.18
Ascaris spp. + *Entamoeba* spp.	1	4.35	0.67-20.50
Triple infection	7	30.43	15.29-50.43
Eimeria spp. +*Strongyles* + *Strongyloides* spp.	1	4.35	0.67-20.50
Eimeria spp. + *Strongyles* + *Entamoeba* spp.	1	4.35	0.67-20.50
Eimeria spp. + *Ascaris* spp. + *Entamoeba* spp.	1	4.35	0.67-20.50
Strongyles + *Entamoeba* spp. + *Strongyloides* spp.	1	4.35	0.67-20.50
Strongyles + *Ascaris* spp. + *Entamoeba* spp.	1	4.35	0.67-20.50
Eimeria spp. + *Strongyles* + *Ascaris* spp.	2	8.70	2.55-27.18
Quadruple Infection	5	21.74	9.83-42.18
Strongyles + *Ascaris* spp. + *Entamoeba* spp. + *Metastrongylus* spp.	1	4.35	0.67-20.50
Eimeria spp. + *Strongyles* + *Ascaris* spp. + *Strongyloides spp.*	1	4.35	0.67-20.50
Eimeria spp. + *Strongyles* + *Entamoeba* spp. + *Strongyloides spp.*	1	4.35	0.67-20.50
Eimeria spp. + *Strongyles* + *Ascaris* spp. + *Entamoeba* spp.	2	8.70	2.55-27.18
Protozoan + Protozoan	1	4.35	0.67-20.50
Double infection	1	4.35	0.67-20.50
Eimeria spp. + *Entamoeba* spp.	1	4.35	0.67-20.50

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Conflict of interest statement

The authors declare no conflict of interest

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