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Prevalence of Gastrointestinal Parasites in Free-Ranging Bantengs (*Bos javanicus*) and Domestic Cattle at a Wildlife and Livestock Interface in Thailand

Nattaya Watwiengkam[1,4], Patinya Patikae[1,4], Pintira Thiangthientham[1], Nuntita Ruksachat[2], Supanun Simkum[1], Kittikom Arunlerk[1], Kamonchanok Chooyoung[3] and Tarid Purisotayo[1,4,*]

[1]Faculty of Veterinary Sciences, Mahasarakham University, Maha Sarakham 44150, Thailand
[2]Department of National Parks, Wildlife and Plant Conservation, Bangkok 10900, Thailand
[3]School of Biodiversity, One Health & Veterinary Medicine, University of Glasgow, Glasgow G12 8QQ, United Kingdom
[4]Veterinary Infectious Disease Research Unit Mahasarakham University, Maha Sarakham 44150, Thailand

([*]Corresponding author's e-mail: tarid.p@msu.ac.th)

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Abstract

Impact of human activities on conservation of endangered species is significant due to the creation of wildlife-livestock interfaces (WLI), which increases the likelihood of disease transmissions. Protected wildlife populations may suffer from diseases caused by gastrointestinal (GI) parasites, potentially impacting conservation efforts. A population of ~100 wild bantengs inhabits the Lam-Pao non-hunting area, which is adjacent to human communities, animal farms and the Pao River, making this WLI suitable for a study of prevalence of GI parasites in the bantengs and domestic cattle. This research examined the prevalence and diversity of GI parasites and explored the associations between the parasitic species identified in the 2 animal groups. Formalin ethyl - acetate concentration technique (FECT) identified overall prevalence of 26.2, 17.4, 53.3 % for both animal groups, bantengs and domestic cattle respectively. The following prevalence of parasitic eggs were identified in the bantengs (N = 46) and the domestic cattle (N = 15) respectively: 1) 8.7 and 46 % of strongyle-type; 2) 4.3 and 6.7 % of *Moniezia* spp.; 3) 2.2 and 0 % of *Fasciola* spp.; 4) 6.5 and 6.7 % of Rumen flukes; 5) 0 and 13.3 % of *Toxocara* spp.; 6) 0 and 6.7 % of *Capillaria* spp.; 7) 0 and 6.7 % of *Strongyloides* spp.; and 8) 0 and 6.7 % of *Trichuris* spp. The combination of coproculture and polymerase chain reactions (PCR) was used to identified common strongyle-type species. We determined *Oesophagostomum radiatum* in 2 cattle (13.3 %) and 3 (6.5 %) banteng samples, *Cooperia onchophora* in 1 (6.7 %) cattle sample and *Ostertagia ostertagi* in 1 (6.7 %) cattle sample. The results present in this study would enable management to proactively strategize and prepare for parasitic diseases that can endanger such a population.

Keywords: Banteng, Cattle, Conservation, Parasite, Prevalence, Wildlife-livestock interface

Introduction

Banteng (*Bos javanicus*) is a wild cattle species that inhabits Southeast Asia, ranging from Myanmar, Thailand, Cambodia, Vietnam and Indonesia. The species has been listed as "endangered" by the International Union for Conservation of Nature (IUCN) red list of threatened species with a global estimate of 4,000 - 8,000 free-ranging individuals; however, the number has been declining [1]. Bantengs, as a large herbivorous species, play an important role in ecosystems. Feeding behaviour of the banteng shapes wild landscapes through plant controls (i.e. grazing) and seed disseminations (i.e. defecation) which were documented to facilitate wildfire prevention [2,3] and litter decomposition [4]. The species has been threatened either directly or indirectly by a range of anthropogenic activities such as habitat loss and/or deterioration, poaching, introduction of exotic plant and animal species, expansion of agricultural activities and farming, unusually prolonged dry seasons [5], and emerging or re-emerging diseases [6]. The latter occurred to be a crucial threat to the species conservation, given the increasing demands for land uses of human activities, which consequently created WLI [7] and thus enhanced likelihood of disease transmissions between the 2 groups of animals. Given such a threat, this warrants the need of strategic actions to curb the declining of wild banteng populations.

In Thailand, the largest population of bantengs has been protected in Huai Kha Khaeng Wildlife Sanctuary located in the west of the country. Other small and fragmented populations were established in the northeast and eastern Thailand [8]. Although the populations are kept in legally protected areas, the overall number of bantengs in Thailand saw an 85 % contraction [9]. Recently, an outbreak of lumpy skin disease (LSD) in the country was first reported in March 2021, initially affecting domestic cattle [6] and later reported in bantengs and wild gaurs (*Bos gaurus*) [10]. Case fatality rate in domestic animals were about one-tenth [10]; however, there was no death confirmed in wild cattle, only 1 LSD-affected gaur died due to fighting (The Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, unpublished data). Despite no fatality reported for LSD-affected wild cattle, the outbreak of such an emerging disease was shown to be a crucial risk for banteng conservation.

GI parasites are ones of important medical conditions that may contribute to diseases and deaths in the protected banteng populations. In some cases, wild bantengs may be a source of parasitic infections for domestic animals or *vice versa*, as well as possessing the potential for zoonotic transmission [11]. Due to the increase of anthropogenic activities at the WLI [7], transmissions of GI parasites between the bantengs and domestic cattle become a concern. Lampao non-hunting area was founded in 1988 at Kalasin province, Thailand. The area possesses the total of 338 square kilometers, approximately 3 of which harbour about 100 free-ranging bantengs in a section known for the local communities as Suan Sa-On (GPS coordinates 16.61345, 103.45910). Suan Sa-On is surrounded by human communities, livestock farms and the Pao River, which make the section suitable for the study of prevalence of GI parasites in the bantengs and domestic cattle at the wildlife and livestock interface. Health status of the population is unknown as such prevalence has never been investigated. In this study we aimed to determine prevalence and diversity of GI parasites and to address associations between parasitic species identified in the 2 groups of animals. The results would provide health status of the legally protected bantengs. Also, this study would allow further studies regarding conservational concerns such as anthelmintic resistance and sensitivity tests, transmissions between wildlife and livestock, and clinical features which would benefit conservation of the species both local and global levels.

Materials and methods

Ethical approval

The works involving animals were approved by the Institutional Animal Care and Use Committee, Mahasarakham University (IACUC-MSU), Thailand, with Approval number IACUC-MSU-32/2021. The study on the legally protected species was authorized by the department of natural parks, wildlife and plant conservation, Thailand (MNRE 0907.4/439).

Sample collection

There were approximately 100 free-ranging bantengs that inhabited Suan Sa-On. These individuals could be divided into 4 subpopulations based on observational data. We conducted on-foot tracking of all the subpopulations and obtained approximately 20 g fresh fecal samples. Local cattle farms located within 3 km from Suan Sa-On were chosen. Fecal samples were collected from the domestic cattle using the same approach as for the bantengs. Minimum sample sizes for bantengs and domestic cattle were estimated to achieve 95 % confidence level and ± 10 % of standard error level, using SPSS Statistics for Windows, Version 29.0 (IBM®, New York, USA). Overall, 46 and 15 fecal samples were obtained from the wild bantengs and domestic cattle, respectively (**Figure 1**). Each fecal sample was kept in a sealed plastic bag and kept in an ice-cooled container (~4 °C). Samples were promptly transferred to the parasitology laboratory, faculty of veterinary sciences, Mahasarakham University, Thailand and preserved at 4 °C until further processing.

Identification of parasitic eggs and statistical analysis

Identification of parasitic eggs was conducted for each fecal sample using formalin ethyl - acetate concentration (FECT) technique described in [12]. The technique allowed the identification of parasitic eggs. Two g of each sample were added to 10 mL of normal (0.9 %) saline water in a 15 mL tube, and centrifugation was done at 2,500 round per minute for 5 min. The supernatant was discarded. The infranatant was kept and added 10 % formalin and ethyl-acetate. Centrifugation was performed at 2,500 round per minute for 5 min, and only the infranatant was kept. The infranatant was fixed in 1 mL of 10 % formalin solution. Identification of parasitic eggs was done under microscopic examination and genera were determined based on morphological features of the eggs. Prevalence was defined as a percent of samples that are infected with a parasite of interest divided by the total number of samples that were examined.

Pearson's chi-square [13] was employed to evaluate statistical differences between the prevalence observed in the wild bantengs and domestic cattle (p-value < 0.05).

Parasitic culture

All samples were used for coproculture. Here, we employed Harada and Mori coproculture technique [14] to allow further specific distinguishability using molecular technique. Each fecal sample was placed onto the central 2-third position of a piece (3×13.5 cm^2) of filter paper (0.34 mm thickness, Whatman® 3MM Chr, UK). The filter paper was put in vertical orientation into a 50 mL tube that contained 10 mL of dechlorinated water, and only the bottom edge of the paper was allowed to touch the water. The tube was sealed and left at room temperature (28 - 30 °C) for 7 days. The technique would allow tropisms of the larvae toward the dechlorinated water, which would later be used to detect the presence of the 3rd stage larvae. The samples were subjected to microscopic examination under magnification levels of 10× and 40× for the purpose of detecting the existence of L3 larvae [15].

DNA extraction and polymerase chain reactions (PCRs)

The larvae obtained from coproculture of an individual sample were used for DNA extraction following the manufacturer's protocols (DNeasy® Blood and Tissue Kit, Qiagen). Quantification and qualification of the DNA materials were done using nano spectrophotometer (Lambda®, PCRmax, UK). The DNA materials yielded from an individual sample were used as templates for each PCR reaction [16] that consisted of 25 µL of master mix (2× ViRed Taq Master Mix, Vivantis, Malaysia); 12 µL of nuclease-free water; 1 µL (20 pg/mL) of DNA templates [16]; and 1 µL (0.2 µM/ 50 µL) of the primers I, II, IV, V and 2 µL (0.4 µM/50 µL) of the primers III described in

Table 1 [16]. The PCR reactions were conducted under the following conditions: 1) Initial denaturation at 94 °C for 5 min; 2) Denaturation at 94 °C for 1 min; 3) Annealing at 60 °C for 1 min; 4) Extension at 72 °C for 2 min; 5) Repeat of steps 2 - 4 for 40 cycles; 5) Final extension at 72 °C for 7 min and kept at 4 °C. Specific identifications of the parasites were made based on sizes of the amplicons that were judged relatively to 100 bp DNA ladder (Vivantis, Malaysia) in 2 % agarose gel electrophoresis in 1× TBE buffer.

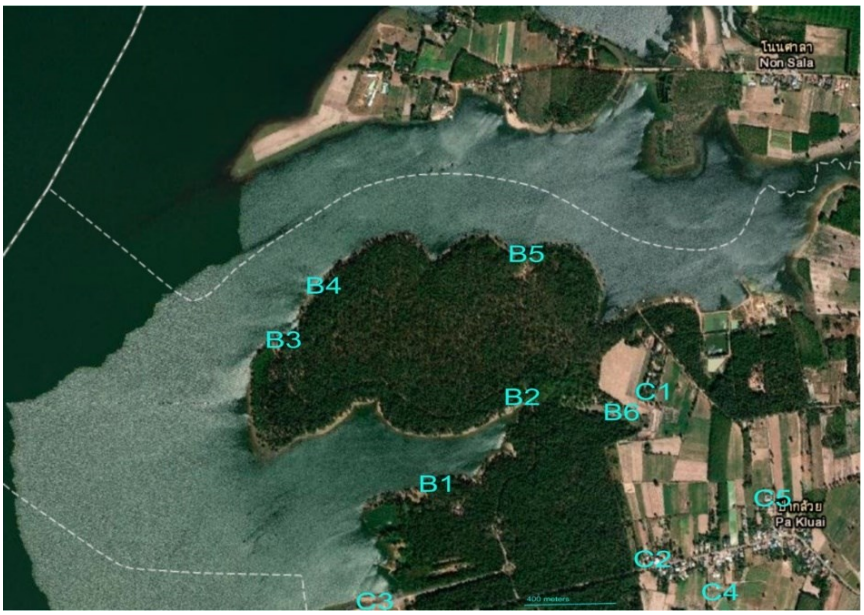

Figure 1 Map of Suan Sa-On illustrating sampling sites of the bantengs (B1 - B6) and domestic cattle (C1 - C5) (modified from Geo-Informatics Center for Thailand). Three banteng samples were collected at the camping site (B1). Three and 11 banteng samples were obtained from mini zoo (B3) and neighboring area (B4), respectively. The remaining fecal specimens were collected from B2 (N = 7), B5 (N = 14) and B6 (N = 8). Fifteen samples of the domestic cattle were obtained from 5 local farms (3 representatives for each of the 5 locations).

Table 1 Primers used in the multiplex PCR (modified from [16]) to allow specific identification of strongyle parasites.

Primers	Parasites	Sequence	Expected size (bp)	Reference or accession number[a]
I - F	*Ostertagia ostertagi*	5'TAAAAGTCGTAACAAGGTATCTGTAGGT	257	[17]
I - R	*Ostertagia ostertagi*	5'GTCTCAAGCTCAACCATAACCAACCATTGG		AF044933
II - F	*Haemonchus placei*	5'CATTTTCGTCTTGGGCGATAT	176	AF343971
II - R	*Haemonchus placei*	5'TGAGACCGCACGCGTTGATTCGAA		AF343971
III - F	*Oesophagostomum radiatum*	5'GCAGAACCGTGACTATGGTC	329	AF344881
III - R	*Oesophagostomum radiatum*	5'GACAAGGAGATCACGACATCAGCAT		AJ006149
IV - F	*Trichostrongylus colubriformis*	5'CAGGGTCAGTGTCGAATGGTCATTGTCAAATA	243	S69220
IV - R	*Trichostrongylus colubriformis*	5'CAGGGTCAGTGGTTGCAATACAAATGATAATT		S69220
V - F	*Cooperia onchophora*	5'TCGATGAAGAGTTTTCGGTGTTC	151	AF343972
V - R	*Cooperia onchophora*	5'TTCACGCTCGCTCGTGACTTCA		AF343972

Note: [a]accession numbers recorded in GenBank.

Results and discussion

Totally, 46 and 15 fecal samples were obtained from the wild bantengs and domestic cattle, respectively. Based on the FECT, the following GI parasites were identified in the bantengs (N = 46) and the domestic cattle (N = 15) respectively (**.**

): 1) Strongyle-type eggs observed in 4 (8.7 %) and 7 (46 %) samples; 2) *Moniezia* eggs observed in 2 (4.3 %) and 1 (6.7 %) samples; 3) *Fasciola* eggs observed in 1 banteng sample (2.2 %); 4) Rumen fluke eggs observed in 3 (6.5 %) and 1 (6.7 %) samples; 5) *Toxocara* eggs observed in 2 cattle samples (13.3 %); and *Capillaria*, *Strongyloides*, and *Trichuris* eggs were identified in a different (6.7 %) sample. The results in the present study showed overall prevalence of 26.2, 17.4, 53.3 % for both animal groups combined, bantengs and domestic cattle respectively, which were much lower than the prevalence in wild animals reported elsewhere [18,19]. Regarding genera diversity, we identified 4 genera in the wild banteng population; whereas, 7 were revealed in the domestic cattle. The results suggested that, in these particular populations, wild bantengs might not be the sources of GI parasites for the cattle. Such finding was opposite to results of previous studies [20,21] and a notion that wild animals were natural reservoirs of GI parasites with little or no clinical effects [21]. Potentially, the results in the present study were limited by the inability to track the bantengs individually that might lead to an individual being sampled more than 1 occasion. The limitation possibly introduced over- or underestimation of the prevalence, which the latter was more likely the case for the present study. Underestimation of the prevalence was also reported when 1 fecal sample [22] or copromicroscopic technique [23] was used, which might reduce sensitivity of the genera identification. Using multiple fecal samples per an individual could increase sensitivity and was suggested [22]; however, this practice might be challenging in wild animal studies. The present study was constrained by a sample size of domestic cattle that was limited by the inclusion criteria that required the presence of these animals within a 3 km radius. With such small sample size, it was plausible that this constraint might potentially lead to an underestimation of the prevalence and/or range of genera diversity [24], as this would subsequently decrease the probability of detecting a less prevalent genus. However, it has been documented that prevalence did not vary consistently across a wide range of host population sizes [25].

Strongyle-type was the most prevalent eggs identified in both the bantengs and the domestic cattle (**.**

), which were consistent with the studies previously done in Thailand [26-28]. Statistical differences between the 2 groups were noted in the prevalence of strongyle-type and *Toxocara* eggs with higher prevalence observed in the domestic cattle. The high incidence in domestic cattle might be resulted by farming practices where the animals were confined to enclosures with a disproportionately high animal

density because such practice would allow re-infections. On the contrary, free grazing of the wild bantengs would disrupt life cycles of the parasites that might contribute to the low prevalence.

As strongyle-type group was the most prevalent based on the FECT, we further determined specific species of these parasites using the coproculture technique in a combination with the PCR (**Figure 2**). Of the 46 and 15 samples obtained from the bantengs and domestic cattle, respectively, 8 and 3 samples were positive for the 3rd stage larvae yielded from coproculture. Three and 2 samples of which, respectively, were positive for at least 1 strongyle parasite that were described in

Table 1. In 2 domestic (13.3 %) samples and all 3 banteng samples (6.5 %), *Oesophagostomum radiatum* was found. Two distinct domestic samples both contained *Cooperia onchophora* (6.7 %) or *Ostertagia ostertagi* (6.7 %). Strongyles were found to have a higher prevalence elsewhere in Thailand among domestic cattle [26-28]. Based on numerous factors, including diversified management practices, feeding regimes and genetic factors, dairy cattle exhibited a higher incidence than beef cattle [28]. All the specimens obtained from domestic cattle, as presented herein, were gathered from the beef cattle, and may have consequently resulted in the low prevalence. Unlike the identification of parasites based on microscopic examination, we demonstrated in this study that the combination between coproculture and PCRs allowed species identification [23,29]. Additionally, such a combination would suit studies in wild animals that samples obtained are often degraded and may be overlooked by microscopic techniques [30,31] attributable to the challenging conditions during sampling processes. We therefore recommend using the combination techniques in further studies that concern parasitic issues in wild animals. The selection of PCR primers remained an additional element that could potentially influence the results of such studies. This highlights the necessity for an establishment of a multiplex methodology that covers the majority of parasites commonly found in wild animals to enable a precise and reliable assessment of the incidence of intestinal parasites in the wild species. The outcomes exhibited in this study represent the investigation of the prevalence of parasites in this wild banteng population, which has been threatened by anthropogenic actions. Thus, it is imperative to consider human activities on host-parasite dynamics to avert the transmission of the parasite from domestic animals to wildlife and *vice versa*. Maintaining a buffer zone at WLI is recommended [32]. However, such an approach may be challenging because the human communities have been long established, and the wildlife habitats have been utilized for tourist activities as observed in the case of the present study. Furthermore, the presence of non-livestock animals can present challenges in the creation of a buffer zone due to their potential for free roaming [11]. Alternatively, as part of the One Health approach to preventing and controlling transmissions, it is advisable to implement public education programs and epidemiological measures in disease prevention [33]. These practices would serve to inform both park personnel and tourists.

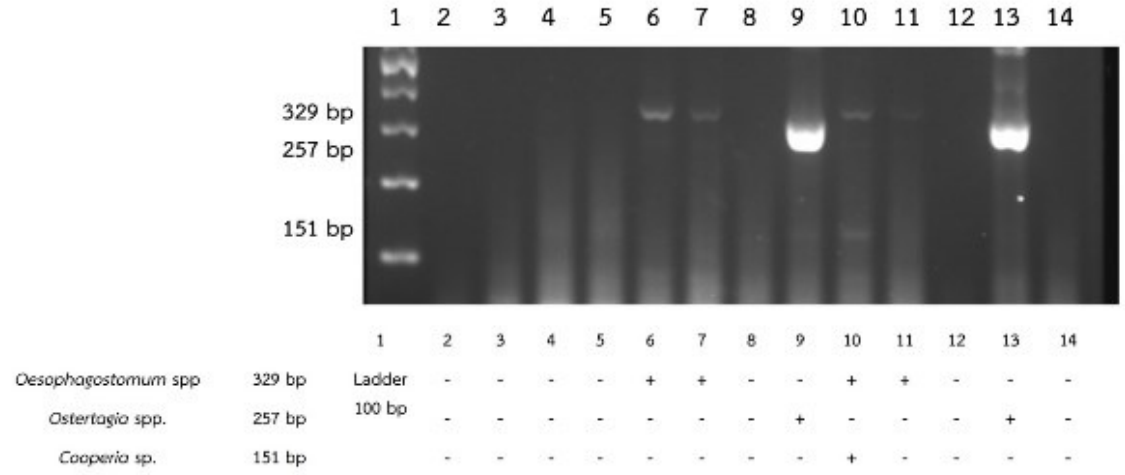

Figure 2 Gel electrophoresis of the amplicons yielded from the multiplex PCR. The positive results were defined based on size-determination relatively to 100 bp DNA ladder and noted with plus signs.

Table 2 Comparison between prevalence of GI parasites identified in the bantengs and domestic cattle using FECT technique.

Parasites	Prevalence				*p*-value
	Bantengs (N = 46)		Cattle (N = 15)		
	No. of infected animals	Percent (%)	No. of infected animals	Percent (%)	
Strongyle-type	4	8.7	7	46.7	0.001[a]
Moniezia spp.	2	4.3	1	6.7	0.718
Fasciola spp.	1	2.2	0	0	0.565
Toxocara spp.	0	0	2	13.3	0.012[a]
Capillaria spp.	0	0	1	6.7	0.077
Rumen flukes	3	6.5	1	6.7	0.984
Strongyloides spp.	0	0	1	6.7	0.077
Trichuris spp.	0	0	1	6.7	0.077

Note: [a]statistical difference (p-value < 0.05).

Conclusions

This study provides information about the prevalence of GI parasites in free-ranging bantengs and domestic cattle at a wildlife and livestock interface in Thailand. The study found that bantengs had a higher overall prevalence, but lower genera diversity of GI parasites compared to domestic cattle, and that some parasites found in bantengs were different from those found in domestic cattle (i.e. strongyles and *Toxocara* spp.). The provision of this data would equip management with the capacity to execute proactive strategies and arrangements to mitigate the impact of parasitic diseases that may pose a threat to the banteng population.

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