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Table of Contents

Medicine and Surgery

Surgical Removal of a Cervical Sialocele in a 9-Year-Old Intact Female Shih Tzu
Matthew Benedict T. Calibo and Ma. Imee M. Macaraig 4

Microbiology

Molecular Detection and Sequence Analysis of Chicken Infectious Anemia Virus from Commercial Chicken Flocks in Select Regions of the Philippines
Fletcher P. Del Valle and Dennis V. Umali 15

Development of a LAMP Simulation and Selection Pipeline to Predict Primer Success
Yuichi Sanekata, Kotetsu Kayama, Taichi Endoh, Daiji Endoh, and Gerry Amor Camer.....26

Comparative Gene Expression Analysis of Immune-Related Cytokines in *Riemerella anatipestifer*-Stimulated Philippine Banaba Native Chicken and Native Duck Embryonic Fibroblasts
Cherry P. Fernandez-Colorado, Mark Joseph M. Desamero, Saubel Ezrael A. Salamat, Gordon Karl Barbour M. Torno, Kane Errol M. Untalan, Kiariza V. Kindipan, John-John R. Fatalla, Ron Carlos R. Linatoc, and Jennelyn Joyce D. Tibar.....39

Parasitology

Toxocara vitulorum-*eimeria spp.* Mixed Infections and Treatment in a 44-day-old Anatolian Black Calf
Alper Ertürk, Merve İder, Onur Ceylan, and Murat Kaan Durgut.....51

Gastrointestinal Nematode Infections of Deer and Sheep in an Agritourism Farm in Bogor, Indonesia
Ridi Arif, Eddy Sukmawinata, Nanis Nurhidayah, Fadjar Satrija, Harimurti Nuradji, Robby Wienanto, and Taisei Kikuchi 59

Pathology

Gross and Microscopic Pathology of Pigeon Paramyxovirus Serotype 1 (PPMV-1) Infection in Racing Pigeons (*Columba livia domestica*) from Luzon, Philippines
Cris Niño Bon B. Marasigan, Ma. Suzanneth Epifania G. Lola, and Dennis V. Umali.....66

Public Health

Monitoring Antibodies against FMD Using ELISA in Vaccinated and Unvaccinated Cattle in Gresik Regency, Indonesia
Rinasti R. Pangesti, Suwarno, Jola Rahmahani, and Dwi K. Lestari..... 75

Systematic Review and Meta-Analysis on the Prevalence of Campylobacter in Poultry in Asia
Fredelon B. Sison, Roderick T. Salvador, and Romeo S. Gundran.....85

Surveillance of *Brucella suis* in Pigs from Selected Slaughterhouses in Luzon, Philippines Using Serological and Molecular Assays
Cheav Chhuon, Ma. Suzanneth Epifania G. Lola, Saubel Ezrael A. Salamat, Aaron Paul R. Serdeña, and Cherry P. Fernandez-Colorado.....96

Zootechnics

Evaluation of *Bifidobacterium sp.* and *Guazuma ulmifolia* Leaf Extract on Quail (*Coturnix coturnix-japonica*): Influences on Feed Intake, Feed Conversion Ratio, and Quail Day Production *Aprinda Ratna Lovela, Widya Paramita Lokapirnasari, Mohammad Anam Al Arif, Soeharsono, Sri Hidanah, Sunaryo Hadi Warsito, Redilla Prasinta, Tiara Hapsari, and Asafarid Andriani*.....105

Short communication

Gastrointestinal Nematode Infections of Deer and Sheep in an Agritourism Farm in Bogor, Indonesia

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Abstract

Background: This study aimed to investigate gastrointestinal nematode infections of Indonesian deer and sheep in an agritourism farm. **Method:** A total of 54 fecal samples were collected from spotted deer (*Axis axis*; n=6), Timor deer (*Rusa timorensis*; n=3) and sheep (n=45) from an agritourism farm in Bogor Regency, West Java, Indonesia. In addition, five fecal samples were collected from five Timor deer enclosures in Bogor city. All samples were subjected to fecal egg and larval identification. Collected larvae were identified by polymerase chain reaction and sequencing. Results: Gastrointestinal nematode infection was higher in sheep than deer. Most infections were caused by strongyle infection in both animals. In this study, *Haemonchus contortus* was successfully sequenced from sheep and Indonesian deer. Our results showed that *H. contortus* isolates from Indonesian deer were placed in a clade with *H. contortus* from other infected hosts like sheep and goats. **Conclusions:** This is the first report of *H. contortus* isolated from Indonesian deer. The parasite control program is necessary to be conducted in both animals, especially considering the high number of sheep infected with nematode in the studied farm.

Keywords

Gastrointestinal nematode; *Haemonchus contortus*; spotted deer (*Axis axis*); Timor deer (*Rusa timorensis*); sheep

1. Introduction

In recent years, as human populations have increased, including in Indonesia, wildlife habitats have been converted to land for agricultural production and human settlement. This situation means that humans and domestic animals become part of a sylvatic cycle [1]. Transmission of pathogens, including parasitic nematodes, between wild and domestic animals can occur directly or indirectly through shared environments and vectors [2]. Previous studies have shown that up to 70% of nematode species are shared between wild and domestic animals and vice versa [3]. Wild deer could potentially act as vectors of anthelmintic-resistant nematodes in cattle, sheep, and other domestic animals [4].

In Indonesia, spotted deer (Axis axis) and Timor deer (*Rusa timorensis*) are considered potential food sources for animal protein [5,6]. Several breeding stations are located close to livestock farms, such as sheep farms. The proximity of deer farms to livestock farms needs to be assessed because of the potential for

cross-infection of parasitic nematodes between these animals. The aim of this study was to identify the gastrointestinal nematodes among deer and sheep that were kept under the same management at an agritourism farm in Bogor Regency, West Java, Indonesia.

2. Materials and Methods

2.1 Sample collections

This study was conducted at an agritourism farm that breeds several animals including spotted deer, Timor deer, and sheep in Bogor Regency, West Java Province, Indonesia. Spotted and Timor deer are bred in a mixed colony shed, while sheep are commercially bred in three flock cages with five capacities per flock for fattening purposes. Deer and sheep were reared at a distance of around 100 m and never grazed at the same location (Fig. 1). None of the animals were treated with anthelmintics.

Fecal samples were collected from a total of six spotted deer, three Timor deer and 45 sheep from August to September 2023. Fresh fecal samples were collected immediately after defecation, placed in plastic bags, and transported to the laboratory for fecal examination. Sampling was carried out three times with seven days intervals. In each sampling event, all deer populations were recorded and sampled from the same individuals, whereas fifteen sheep fecal samples were not taken from the same individuals at all sampling events due to trade factors. So, nine deer in an agritourism farm were sampled in three independent replicates in this study. For comparison, fecal samples of five Timor deer kept at a deer breeding facility in Bogor, away from the livestock farms, were also sampled once.

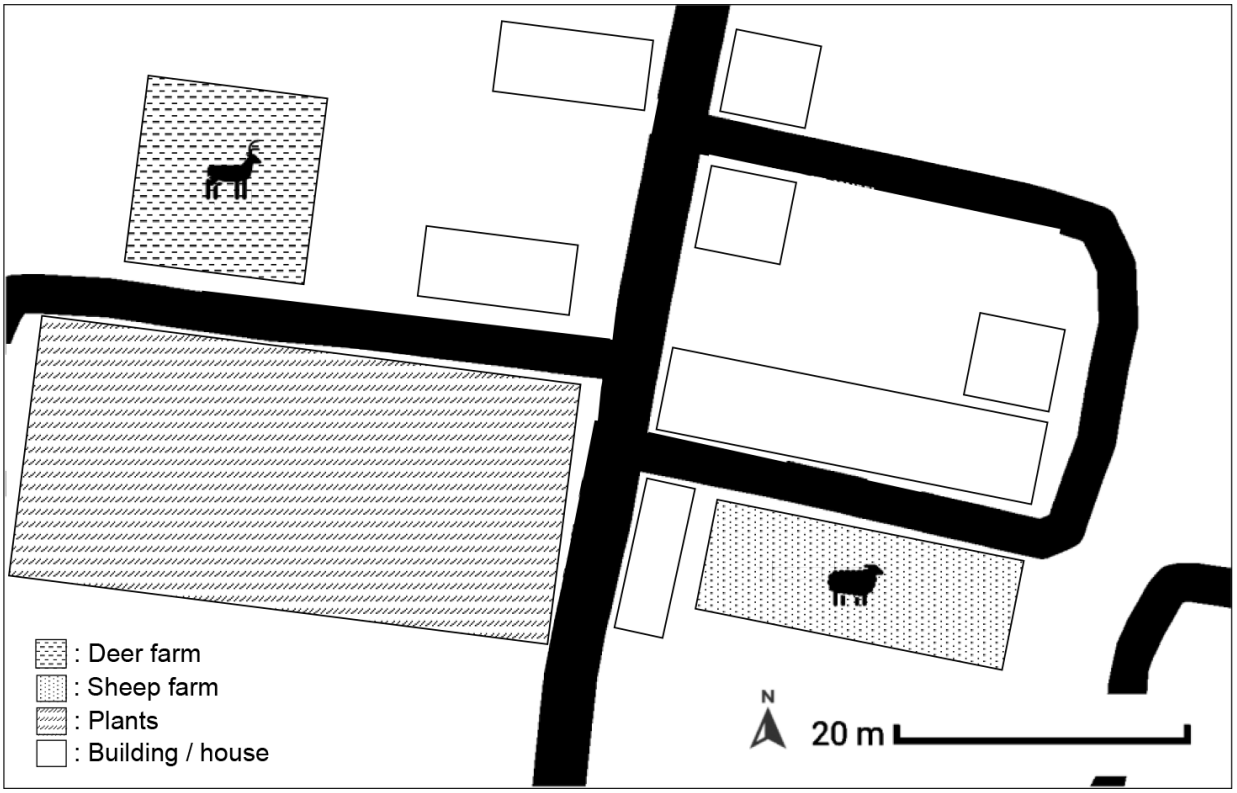

Fig. 1. Map of deer and sheep samples in the study area of agritourism farm.

2.3 Fecal egg count and larvae identification

Fecal egg count (FEC) was performed by using the McMaster method according to Zajac and Conboy [7] with a sensitivity of 50 eggs per gram (EPG) of feces. The level of infection was categorized from FEC results as mild (<250 EPG), moderate (250-1000 EPG), and severe (>2000) infection based on Taylor et al. [8]. When the sample was negative by the McMaster method, 10-15 mL of residual suspension was further examined by direct flotation [8]. Direct flotation gave only a qualitative result (positive/negative), and positive infections from the flotation method were included as mild infections. Furthermore, infective L3 larvae were obtained from positive samples; feces samples were mixed with vermiculite and incubated at room temperature with moist condition for seven days [9]. Nematode larvae were collected using the Baermann method on the seventh day and identified to the genus level based on morphology [8].

2.3 DNA extraction and sequencing

DNA extraction from the infective L3 larvae was performed according to Kikuchi et al. [10] with minor modifications. Briefly, the individual L3 nematodes were washed with sterilised phosphate-buffered saline (PBS) and transferred to a 0.2 mL tube containing 10 µl of lysis solution (9 µl Direct PCR [101-T, Viagen, CA, US], 0.5 µl of 20 mg/mL Proteinase K [P8107S, BioLab, UK] and 0.5 µl of 1 M dithiothreitol [PTE1617, Wako, Osaka, Japan]). Subsequently, the lysates were incubated at 60 °C for 1 h and then at 95 °C for 10 min. To identify the nematode species, the universal primers 988F and 1912R were used to amplify a partial region of the 18S ribosomal RNA gene (Table 1). Nematodes identified as *Haemonchus* based on morphology were confirmed the species using species-specific primers as described by Mafuna et al. [11]. Polymerase chain reaction (PCR) amplicons were sequenced on an ABI 3500XL Genetic Analyzers (Applied Biosystems) using the BigDye Terminator v3.1 kit. The sequence results were then compared with sequences in the nt database of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi). Nucleotide sequences were aligned using ClustalW and maximum likelihood phylogenetic trees were constructed using MEGA version 11.0.13. The sequences of the six *H. contortus* isolates were deposited in the NCBI GenBank database.

2.4 Statistical analysis

The proportion of infected hosts and the mean of FEC between deer and sheep at each sampling event on the agritourism farm were calculated using Microsoft Excel version 16.70.

3. Results

In general, the rate of nematode infection in deer were lower than sheep at the studied agritourism farm (Table 2). The microscopic examination of the individual fecal samples revealed that all of deer showed negative at each sampling event. Nevertheless, nematode eggs were consistently detected from one spotted deer (11.1%) with mild infection (<50 EPG) in every sampling after being tested with flotation technique. Then, half of the selected sheep (53.3%; 24/45) revealed nematode infection with various levels, whereas 46.7% (21/45) showed negative infection. Particularly, egg infestation was observed in 26.7% (12/45), 24.4% (11/45), and 2.2% (1/45) of sheep with mild, moderate, and heavy degree, respectively. Based on egg morphology, we found that nearly all infected animals were manifested with strongyle eggs. Mix infection

Table 1. Summary of primers used in the current.

Primer pairs	Primer sequence (5'-3')	Targeted regions (amplicon size)	References
988F	5′-CTCAAAGATTAAGCCATGC-3′	18S ribosomal RNA (962 bp)	[10]
1912R	5′-TTTACGGTCAGAACTAGGG-3′		
HcBotuF1	5′-TGTCGAACACGAAACTCGTC-3′	*H. contortus* (260 bp)	[11]
HcBotuR2	5′-TGTGTCTCTACCGCCCGAGT-3′		

(strongyle, *Trichuris*, and cestode) occurred in a sheep with a heavy infection (5100 EPG), and a single infection of Trichuris was identified in three sheep with mild infection. In addition, like infected deer on the farm, four of five Timor deer in the breeding facility were infected with mild infection (<50 EPG) of strongyle eggs.

In this study, a total of six L3 stage *H. contortus* were isolated and identified from a spotted deer (RMT001A and RMT001B) and two sheep (DMT001A and DMT002A) that bred in the agritourism farm, and a Timor deer (RC001A and RC001B) in breeding facility. *Haemonchus contortus* were successfully identified from those six nematodes by PCR (Fig. 2). Nevertheless, 18S rRNA gene marker failed to be sequenced due to insufficient amount of amplicon concentration. The phylogenetic analysis showed that *H. contortus* isolated from Indonesian deer were clustered into one clade with *H. contortus* from other infected hosts like sheep and goats (Fig. 3).

Table 2. Proportion of gastrointestinal nematode infection in deer and sheep in an agritourism farm.

Sampling event	Host	n[a]	Infected host[b] (%)	Fecal egg count (eggs per gram)	
				Mean	Range
Sampling 1	Deer	9	1 (11.1%)	0	0
	Sheep	15	10 (66.7%)	477	0-5100
Sampling 2	Deer	9	1 (11.1%)	0	0
	Sheep	15	8 (53.3%)	160	0-1200
Sampling 3	Deer	9	1 (11.1%)	0	0
	Sheep	15	6 (40.0%)	194	0-1500

[a] Feces samples were taken from the same individual of deer (N = 9) at all sampling events, whereas feces samples from sheep were taken from different individuals (N = 45).

[b] Infected hosts were determined based on positive result from fecal egg count and flotation test.

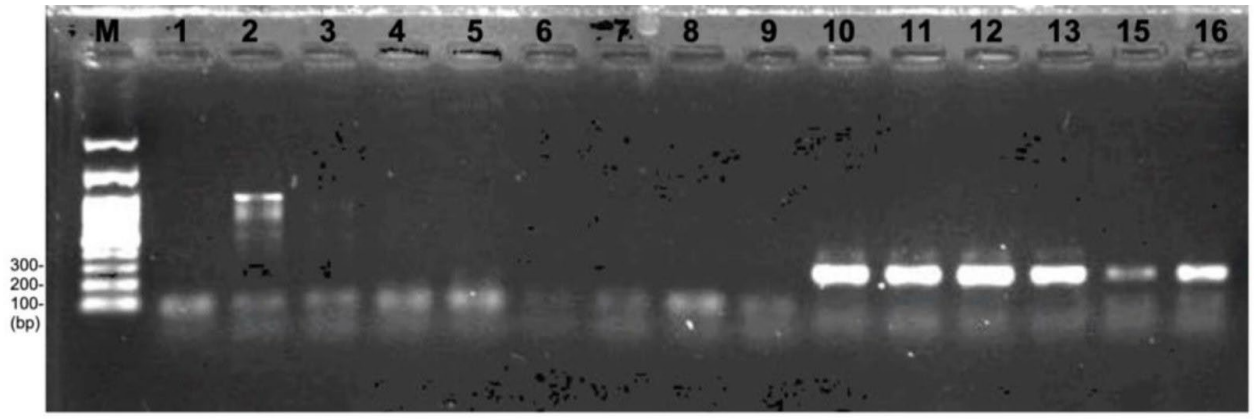

Fig. 2. Agarose gel electrophoresis of PCR-amplified products using 18S rRNA and H. contortus specific primers. Lane 1-6: PCR-amplified products using 18S rRNA primers from isolate RMT001A, RMT001B, DMT001A, DMT002A, RC001A, and RC001B, respectively, lane 7-9: PCR-amplified products using 18S rRNA primers from unknown isolate, lane 10-16: PCR-amplified products (260 bp) using H. contortus specific primers, and lane M = 100 bp DNA ladder.

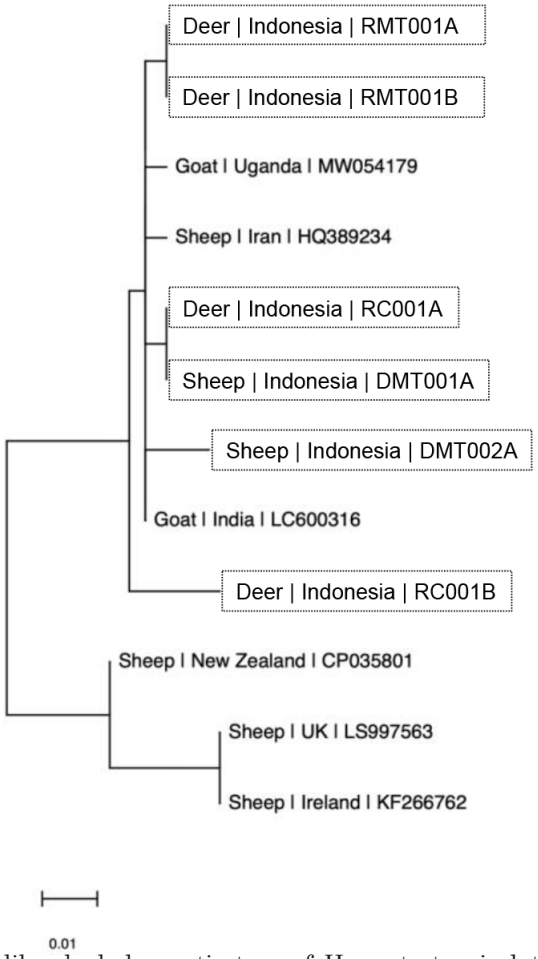

Fig. 3. A maximum-likelihood phylogenetic tree of H. contortus isolates. H. contortus were obtained from a spotted deer (RMT001A and RMT001B) and two sheep (DMT001A and DMT002A) at the agritourism farm, and from a Timor deer (RC001A and RC001B) in a breeding facility. The Indonesian isolates are indicated in the box that provides the following information: host, country, and isolate name. The following information is also provided for each isolate from NCBI database: host, country, and accession number.

4. Discussion

In this study, we found that strongyle infections were the most prevalent in sheep with different levels of infection. Close contact between livestock and wildlife, including deer, may increase the risk of cross-transmission of gastrointestinal parasites [12]. Previously, *H. contortus* was identified as the common species circulating in both sheep and roe deer in south-western France [13]. Here, we succeeded in isolating *H. contortus* from spotted deer on the studied farm. Since the infestation of gastrointestinal nematodes is high in the sheep population, we assumed that deer infection in the agritourism farm could be transmitted indirectly from sheep. Moreover, many nematode species belonging to the sheep strongyle parasite group such as *Haemonchus* spp. and *Trichostrongylus* spp., are important in animal and public health [14].

To our knowledge, organic fertilizers derived from sheep and cattle dung are commonly used for vegetable and grass farming in the studied area. Sheep and deer may have recurrent and accumulative parasite infections when they are fed contaminated grass. Previous studies have reported that contaminated grass for animal feed that is cultivated by using organic fertilizer as growth promoters may have a risk of carrying infective stage intestinal parasites [15], and *Trichostrongylus* infected humans may occur due to consuming contaminated fresh vegetables [16]. So, further study is needed to evaluate the indirect factors that may contribute to the transmission of gastrointestinal nematodes. Our analysis

was limited to using a single gene marker to determine the relationship between sequenced isolates. Nevertheless, *H. contortus* isolates from Indonesian deer were placed in a clade with another infected host like sheep and goats. Furthermore, since *H. contortus* became the most prevalent gastrointestinal nematode in sheep and deer, controlling programs such as anthelmintic administration should be conducted for both animals.

5. Conclusions

This is the first study to show infection with *H. contortus* worms in Indonesian deer. The results of this study suggest that parasite control in sheep farms is particularly necessary to prevent cross-infection into deer populations. In addition, further molecular analysis is useful to confirm clonal transmission between the two animals and gain insight into the characteristics of *H. contortus* in sheep and Indonesian deer.

Author Contribution

Conceptualization, ES, RA, and NN; Methodology, RA, ES, NN, and RW; Investigation, RA and ES; Writing-Original Draft, ES and RA; Writing-Review & Editing, ES, RA, NN, HN, FS, RW, and TK; Funding Acquisition, ES; Resources, ES and R A ; Supervision FS and ES.

Ethics Approval and Consent to Participate

This study was conducted under the approval of the Ethics Commission at National Research and Innovation Agency, No. 066/ KE.02/ SK/04/2023.

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Conflict of Interest

The authors declare no conflict of interest.

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