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RSST012 - COPROLOGICAL AND MOLECULAR INVESTIGATION OF ANIMAL PARASITE IN COW STOOL IN KUALA PILAH, NEGERI SEMBILAN

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ABSTRACT

Animal parasites is one of the significant threat to cattle productivity and public health due to their potential zoonotic risks. This study investigated the presence of *Toxocara* spp., *Cryptosporidium* spp., and *Fasciola* spp. in cattle from three farms in Kuala Pilah, Negeri Sembilan, using both coprological and molecular methods. A total of 30 faecal samples were examined through direct smear, formalin-ether sedimentation and trichrome staining for coprological analysis. Additionally, Polymerase Chain Reaction (PCR) amplification was performed using each parasite-specific primers to detect the DNA. Coprological results revealed *Toxocara* spp. (40%), *Cryptosporidium* spp. (33.33%), and unidentified animal parasites (13.33%), with no *Fasciola* spp. eggs observed. However, PCR did not yield amplification of all the targeted parasite DNA, which may have been due to low infection intensity, the presence of PCR inhibitors, or limitations in primer sensitivity. Interviews with farm managers revealed that deworming practices were inconsistent and management systems varied across farms, including both free grazing and confined feeding conditions, which may contribute to parasite transmission. These findings indicate a possible low prevalence or subclinical fascioliasis among cattle in the study area. To improve detection accuracy, future studies should incorporate validated primers, synthetic DNA as positive controls, and PCR additives. Regular deworming and improved farm hygiene practices are also recommended to manage parasitic risks more effectively.

Keywords – animal parasites, Toxocara spp., Cryptosporidium spp., Fasciola spp., ITS2 region

INTRODUCTION

Animal parasitic infections in cattle are a persistent challenge to livestock productivity and public health. Gastrointestinal parasites such as *Toxocara* spp., *Cryptosporidium* spp., and *Fasciola* spp. cause significant economic losses as well as possible zoonotic transmission. These parasites can reduce milk production, hinder growth and put animals at risk for various illnesses (Caravedo & Cabada, 2020). The prevalence of parasites can be directly impacted by the significant variations in farm management practices in Malaysia such as grazing systems, hygiene routines and deworming schedules. *Toxocara* spp. mostly infect carnivores but also can be found in ruminants and act as carriers during the parasite life cycle which posing risks of visceral or ocular larva migrans in humans (Centers for Disease

Control and Prevention, 2019a). *Cryptosporidium* spp. is an apicomplexan protozoan which causes cryptosporidiosis in calves and poses health threats to immunocompromised individuals (Centers for Disease Control and Prevention, 2019c. While *Fasciola* spp., are important veterinary and medical trematodes with *F.hepatica* and *F.gigantica* both reported in Southeast Asia, including Malaysia (Fürst et al., 2012).

Until these days, limited reports have been recorded regarding animal parasites in Negeri Sembilan, despite numerous studies being conducted in other states across Malaysia. Hence, the risk of parasitic infection in this state remains unknown. This research aims to identify research gaps on the identification and occurrence of parasitic disease in Negeri Sembilan, utilising cross-sectional surveys from several cattle farm. A coprological examination detects parasite eggs in stool samples observed under the microscope by using three different method which is direct smear, formalin-ether sedimentation and trichrome staining to enhance the detection and identification of parasitic organisms (Thakre et al., 2016). Molecular techniques like the nested polymerase chain reaction (PCR) are also used to target the internal transcribed spacer (ITS) of the gene since they have a higher sensitivity for identifying the parasite gene (Verweij & Stensvold, 2014). This research will help farmers and policymakers develop targeted control strategies, such as improved pasture management, snail control initiatives, and deworming methods, which will lessen the disease's impact by identifying high-risk areas and infection trends. In the end, it can give researchers and practitioners information about the parasitic threat in Negeri Sembilan's cow farms.

OBJECTIVE

This study aims to investigate the occurrence of parasitic infections in Kuala Pilah, Negeri Sembilan. The first objective is to carry out coprological examinations of cows' faeces to detect parasites eggs. Second objective is to perform stool DNA extraction and carry out polyerase chain reaction (PCR) to detect parasites gene in stool samples.

METHODOLOGY

30 stool samples were taken from three selected farms early in the morning. To detect parasite eggs based on morphological features, coprological examination was carried out using the direct-smear and sedimentation method. Trichrome staining was used to improve the visualization of parasite structures and promote protozoan identification (Centers for Disease Control and Prevention, 2019b). To improve the sensitivity and specificity for identifying parasite infections, molecular detection was conducted using PCR amplification that targeted the 5.8S rDNA and ITS2 regions and gel electrophoresis was utilized to verify product size (Verweij & Stensvold, 2014). The recorded data were analysed where the occurrence of parasite infestation in cattle using coprological and molecular methods calculated as percentage value. The overall occurrence (in %) at the animal level were calculated by dividing the total number of cattle.

RESULTS AND DISCUSSION

Based on the coprological result, *Toxocara* spp. was the most prevalent parasite, present in 40% (12/30) of the samples, followed by *Cryptosporidium* spp. in 33.33% (10/30) of samples (Table 1 and 2). Four samples (13.33%) contained unidentified parasite eggs that

could not be classified due to morphological complexity (Table 1 and 2). The presence of these parasites, especially zoonotic species such as *Toxocara* and *Cryptosporidium*, underscores their potential impact on animal health, farm productivity, and public health. This study highlights the need for regular parasitological monitoring, improved farm management, and further taxonomic or molecular analysis to identify other potentially important animal parasites.

Table 1: Coprological findings of parasite species found in 3 farms

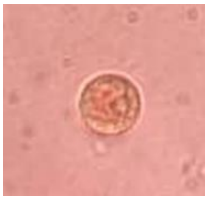
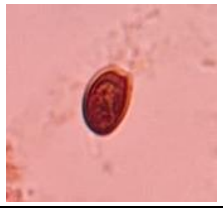
Species	***Toxocara* spp.**	***Cryptosporidium* spp.**	**Other animal parasite**
Image under 100x magnification			

Table 2: Number of parasite species found in 3 farms

Farm/Parasite	***Fasciola* spp.**	***Toxocara* spp.**	***Cryptosporidium* spp.**	**Animal parasite**
Farm A	0/10	4/10	6/10	0/10
Farm B	0/4	1/4	1/4	0/4
Farm C	0/16	7/16	3/16	4/16
Total	0/30	12/30	10/30	4/30

According to molecular findings, PCR amplification targeting the universal 16S rRNA gene of *Eubacteria* confirmed good DNA extraction quality and optimal PCR conditions (Hong et al., 2024). However, amplification for *Fasciola* spp. (expected size: 1000 bp) failed despite repeated standard and nested PCR attempts, suggesting parasite absence or low parasite load in the sampled cattle (data not shown). For *Cryptosporidium* spp., amplification of a 655 bp fragment produced visible bands, but identical amplification occurred in negative controls, indicating primer contamination and made the results unreliable. These molecular results were consistent with coprological observations for *Fasciola* spp. but inconclusive for *Cryptosporidium* spp., highlighting the need for validated molecular protocols to ensure accurate parasite detection.

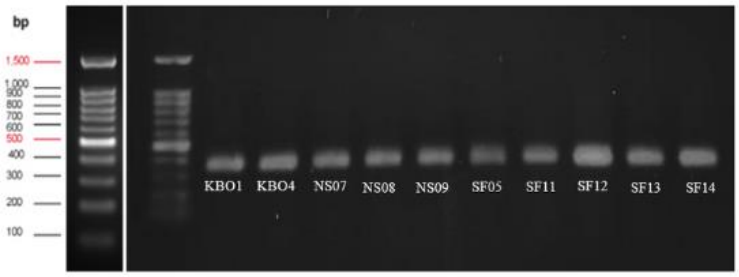


Figure 1: Amplified PCR product of the *Eubacteria* observed on gel electrophoresis

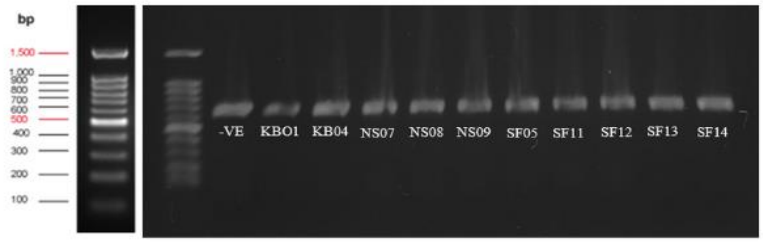


Figure 2: Amplified PCR product of the *Cryptosporidium* spp. observed on gel electrophoresis

CONCLUSION

In conclusion, microscopic analysis revealed various gastrointestinal parasites, with 40% of samples positive for *Toxocara* spp., 33.33% for *Cryptosporidium* spp., and 13.33% for other unidentified species. PCR results did not detect *Fasciola* spp. DNA, but successful amplification of *Eubacteria* DNA confirmed the reliability of the extraction and reagents, suggesting a true absence rather than a technical error. These findings indicate that cattle in the area continue to be exposed to parasitic infections, underscoring the need for routine surveillance, improved diagnostic tools, and regular deworming programs. Enhancing molecular detection through the use of positive controls, PCR additives, and validated primers will further strengthen parasite monitoring and control strategies in livestock across Malaysia.

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