Molecular verification of the intestinal parasites among two breeds of cow from Sagaing Region

Nyein Thi Aung[1], Myo Pa Pa Thet Hnin Htwe Aung [2], Eiji Nagayasu[3],Nyein Nyein Hlaing[4]

Abstract

Livestock farming, particularly rearing of two breeds of cattle (i.e dairy and ploughing cattle) is traditionally practiced by rural people in Myanmar like in other developing countries. Gastrointestinal (GI) parasitic infection is a serious issue in cattle management. The routine diagnosis of GI nematode infections is traditionally based on counting their eggs in a microscopic chamber using a faecal flotation method. This provides little information on the infecting species. Agar plate culture is superior to routine microscopic examination in detection of *S. stercoralis* and hookworms. Thus combination of methods yielded more different types of intestinal nematodes that could rarely be detected by microscopy alone. Moreover, PCR-based methods developed for the identification and detection of various parasite species. Thus this study was performed on specific identification of various cow intestinal parasites by molecular technique of PCR and sequencing, in addition to the microscopy and culture techniques, among two breeds of cows in Sagaing region. In the present study consisting common nematodes by using sugar floatation method and agar plate culture. Moreover, molecular identification by PCR and subsequent sequencing of 18SrDNA marker revealed nine species of intestinal nematodes including *Trichostrongylus colubriformis, Paroiqolaimella micrura, Haemonchus contortus, Zoniolaimus mawsonae, Fictor stercorarius, Rhabditis* sp., *Necator americanus, Ancylostoma caninum,* and *Panaqrolaimus davidi* among the two breeds of studied cattle. The present study highlighted the essential role of molecular techniques in addition to the conventional diagnostic methods for identification down to the species level of the parasites.

Keywords: Gastrointestinal parasites, sugar floatation and culture method, molecular identification

Introduction

Livestock play an important role in many communities in terms of socio-economic development and contribute towards household food and nutritional security, especially among the rural communities. Rearing of dairy cattle in the country is for milk and meat production. Zebu (ploughing cattle) are predominantly used for soil preparation in the cultivation of rice as well as production of curd.The effects of GI parasites may vary with age, sex of cattle, nutritional condition, and severity of infection (Gunathilaka *et al*.,2018). These infections are rarely associated with high mortality of cattle. However, their effects are usuall characterized by lower outputs of animal products, byproducts, manure, and traction, thereby affecting the contributions of cattle in ensuring food security, especially in developing countries. Ruminants may be infected by endoparasites, especially nematode through grass feed that contains worm larvae or eggs. Common cow nematode worms include Strongylidae genera (*Haemonchus, Ostertagia*, *Cooperia, Nematodirus*, *Bunostomum, Strongyloides*, and *Oesophagostomum*)as well as *Capillaria* spp. and *Ascaris* spp. (Nurcahyo et al.,2021) According to the previous study, (Nyein Thi Aung, 2018) the cattle from Sagaing Township were found to be infected with nematodes *Trichuris* sp. ,*Strongyloides* sp. *Trichostrongylus* sp. *Ascaris* sp. *Nematotida* sp. and common cestodes *Fasciola hepatica and Fasciola magna* by using faeces samples and sugar floatation method. A direct microscopy examination of faeces is a simple and rapid test for the diagnosis of intestinal parasitic infection. However, microscopy alone cannot differentiate between the hookworm species and other similar species like strongyloid

[1] 4PhD (Research) Student, Department of Zoology, Yangon University
[2] Professor and Head, Department of Microbiology, University of Medicine, Taunggyi
[3] Associate Professor, Division of Parasitology, Department of Infectious Diseases, Faculty of Medicine, University of Miyazaki, Japan
[4] Professor and Head, Department of Zoology, University of Dagon, Yangon

nematodes such as *Trichostrongylus* spp. and *Oesophagostomum* spp. because all the eggs are morphologically similar.

Thus various coproculture techniques such as Harada-Mori culture (HMC), agar plate culture (APC) and modified APC (MAPC) techniques are employed for morphological characterization of adult and larvae *Anchylostoma duodenale* and *Necartor americanus* and *Strongyloides* spp.(Nongmaithem et al., 2019). Molecular tools can overcome many of the limits of larvae Culture by their higher sensitivity and specificity and better respond to the challenges. Thus the present study aims to perform specific identification of various cow intestinal parasites by molecular DNA technique of PCR and sequencing, in addition to the microscopy and culture techniques, among the two breeds of cows (i.e dairy and ploughing cattle) from Sagaing Region.

Material and Methods

Materials for agar plate culture of parasites

Balance , Stirring device (Wooden applicator) , Weighing spoon, Agar powder (Sigma-Aldrich), Petridish 6mm, Petridish 10mm, adhesive tape, Glycerol (25%),Vinyl tape, Pick up needle , Stereomicroscope, Digital camera

Materials for polymerase chain reaction (PCR)

Water bath , Thermal cycler (applied Biosystem), Electrophoresis apparatus (Mupid- 2 plus), UV Transilluminator (Bio-Rad, Inc), Vacuum (Millipore)(AS One), Spectrophotometer (Thermo Scientific), Micropipettes: 0.5-20 µl, 20-50 µl, and 100-1000 µl , Sterile disposable micropipette tips, Microcentrifuge tubes: 1.5 and 0.5 ml , Gloves .

Materials reagents and chemicals for DNA sequencing

Proteinase K, Dithiotheritol (DTT), Direct PCR (tail) (VIAGEN#101-T), G Taq polymerase (Promega) 15µl, primer Forward ENM173 (5' CTCAAAGATTAAGCCATGC 3'), primer Reverse ENM 174 (5' TTTACGGTCAGAACTAGGG 3') for nematode 18S rDNA marker (Nagayasu *et al*.,2017), Distilled water , dNTPs, 2X Buffer for KoDFx Neo, KoDFx Neo, Template DNA, Sam solution , X-Terminator solution , BigDye X-Terminator Purification Kit(Applied Biosystem).

Preparation of faecal samples for identification and egg count of parasites

In paper cup, 3 grams of faeces were mixed with 50ml of sugar solution and stirred well. The mixtures were poured through a strainer into another paper cup. The strained sample was poured from the paper cup into a test tube that was secured in an upright position. The test tubes were filled up to the top so that when a cover slip was put onto the test tube, the fluid will be just in contact with the cover slip,but not spill over. After 20 minutes, the cover slip was carefully lifted straight up and gently placed onto the center of a microscope glass slide (WHO,1991).The slides were then examined under the compound light microscope at 100X and 400X manifications.

Agar plate culture method for identification

Fresh stool sample of about 2 g was taken and put in the middle of a 6mm and 10mm plates. About 15ml of 25% glycerol was poured onto the agar plates and the lids were sealed tightly with vinyl tape. Agar plates were incubated at room temperature (approximately25 °C) for 4days.Then,they were checked daily under a stereomicroscope to detect any hatched nematodes from eggs in the faecal samples.

Specimen lysis and DNA extraction

Larvae or adult stages of nematodes found on the agar plates were picked up by a worm picker (fine needle made from tungsten) into polymerase chain reaction (PCR) tubes containing 10 µl of worm lysis solution that was prepared by mixing 0.5 µl of proteinase K (> 600 mAU/ml solution, catalog no. 19131, Qiagen), 0.5 µl of 1 M dithiothreitol (DTT), and 10 µl of DirectPCR Lysis Reagent (Tail) (catalog no. 101-T, Viagen Biotech Inc.).The nematodes were lysed by incubation at 60 °C for 20 min.They were then incubated at 95 °C for 20 min to inactivate proteinase K. The tubes containing worm lysate were stored at −30 °C until DNA extraction

Table 1. 18S rDNA PCR amplification reactions of nematodes from cow fecal samples from Sagaing Region

No.	Reagents	1 sample(µl)	100 samples(µl)		PCR condition
1	Water dH_2O	4.5	450		94°C x 2min (denature)
2	2X Buffer for KoDFx Neo	12.5	1250		94°C x 10sec (denature)
3	2mM dNTPs	5.0	500		55°C x 30sec (annealing)
4	Forward (ENM 173) 10uM	0.75	75		68°C x 1min (extension)
5	Reverse (ENM 174)10uM	0.75	75		68°C x 7min (final)
6	KoDFx Neo	0.5	50		Keep at 4°C
Total		**24**	2400		40 cycles
7	DNA template	1	24		
Total in each PCR tube			25µl		

Agarose gel electrophoresis of PCR product

Amplified products were separated by agarose (1.5%, wt/vol) gel electrophoresis in Tris-acetate buffer (0.5X TAE) and visualized with a UV transilluminator (Bio-Rad, Inc.). Firstly,0 .75 gram of 1.5% agarose gel powder was weighed and put into the bottle containing 50 ml of 1X TAE buffer and heated in the microwave oven until the agarose powder was totally dissolved.The bottle was shaked for a while until all powders stick to the bottle wall was dissolved. Then the gel solution was cooled down to 60 ˚C and poured into the casting tray containing comb in the correct position and checked for the air bubbles. The gel was allowed to solidify at room temperature for 30 minutes. Then the comb was removed carefully .The PCR products were prepared by mixing 5 µl of sample and 1 µl of 6 X loading dye on a paraffin paper. Then the gel tray with gel was carefully put into the electrophoresis tank and sufficient TAE buffer was added to cover the gel to the depth of about 1mm. Each of the samples (including positive control and negative control ,6 µl each) was added into separate wells by a micropipette by taking care not to mix the samples from each well. The 100 base pair molecular weight marker DNA ladder (5 µl) was mixed with 1 µl of loading dye and put into the first well. The tank was covered with the lid and the electrode leads were attached correctly to the power supply.The power was set up to 100 VDC and the electrophoresis was performed for 30 minutes. The power was turned off when the Bromophenol Blue loading dye

had migrated in the gel to a distance of half length of the gel tray which is sufficient for separation of DNA fragments. PCR product DNA bands in the agarose gel were visualized under UV light transilluminator and fluorescence imaging, and recording were done by Bio-dot gel documentation system . Interpretation of the results was made on the size of the PCR bands by comparing them with 100 base pair DNA ladder.

Purification of PCR products (partial 18S rDNA)

For purification of PCR products, plastic pipes were assembled and, pressed strongly the plate on Micropile and the wells were prepared and 20ul of PCR products were added into the wells and then, wait for 20 minutes. Subsequently, 50ul was added into each well and wait for another 20 minutes. The wells were checked for dryness and then the vacuum was stopped and the pipes were disconnected. Water (20ul) was added into each well, covered with plastic and put on a plate shaker for 2 minutes. All contents (≈20ul) of purified PCR products were transferred into new PCR tubes.The purified PCR products were measured for DNA concentration by NanoDrop spectrophotometer .

Purification for Sequencing by BigDyeXTerminator Purification Kit (Applied Biosystem)

About 45µl of Sam solution was added into each tube and 10µl of X-Terminator solution was added into each tube. The tubes in the container were sealed by tape and placed in micromixer and run at high speed and set for 30minutes. Then the tubes were covered with an aluminum foil to prevent the light and kept in a cold room. Purified PCR products were sent to the sequencing room. DNA sequencing was done by Sanger DNA sequencing method.

Sequencing PCR

According to their respective results, PCR products were diluted with water to become 25ng/ul for sequencing. The followings are sequencing PCR reactions and conditions.

Sequencing of partial 18SrDNA: PCR Components and Conditions

	Reaction		PCR Condition	
	For 1	For 160	**94°C x 2min**	
			(denature)	
1. dH_2O	6.768	1082.88	**94°C x 10 sec**	
sequencing			**(denature)**	40cycles
2. 5x Buffer	1.800	288.00	**55°C x 30 sec**	
			(annealing)	
3. BigDye	0.400	64.00	**68°C x 1min**	
Terminator			**(extension)**	
4. Seq primer (**100µM**)	0.032	5.12	**68°C x 7min**	
5. template DNA	1.000	160.00	**(final extension)**	
Total (**µl**)	10.000	1600.00	**Keep at** 4°C	

Forward and Reverse Cycle Sequencing of PCR Products (160 reaction)

1600

800**µl** + 2.56ul of **ENM 173 (100µM)** → 802.56 **µl**

800**µl** + 2.56ul of **ENM 174 (100µM)** → 802.56 **µl**

9ul + 1ul of template DNA per reaction (10**µl)**

Purification of sequencing plate was done by SAM solution and X-Terminator solution.

Results

Table 2. Intestinal helminthes detected as ova in faeces of dairy cattle at Kone village

Sr. No.	Types of Helminthes	Infected cattle number(n=50)	Infected %	Egg count
1.	*Fasciola hepatica*	18	36	15
2.	*Strongyloides* sp.	12	24	7
3.	*Trichuris* sp.	10	20	6
4.	*Trichostrongylus* sp.	6	12	4
5.	*Ascaris* sp.	6	12	3
6.	*Fasciola magna*	3	6	2

Table 3.Intestinal helminths detected as ova in faeces of ploughing cattle at Myin Mhwe village

Sr. No.	Types of Helminthes	Infected cattle number (n=42)	Infected %	Egg count
1.	*Ascaris* sp.	29	58	89
2.	*Trichostrongylus* sp.	15	30	14
3.	*Trichuris* sp.	11	22	10
4.	*Strongyloides papillosus*	4	8	2
5.	*Fasciola hepatica*	1	2	1

Coproculture method results

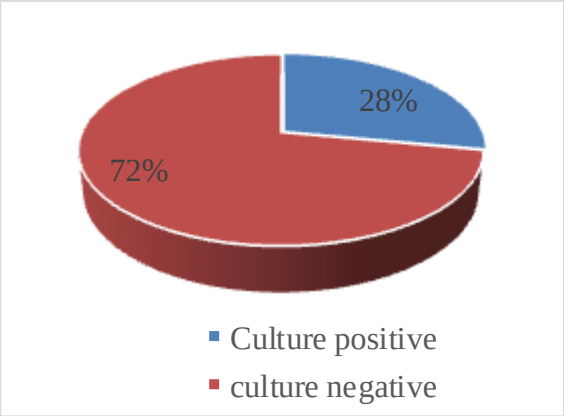

Figure 1. Agar plate culture positivity rate for the intestinal helminthes

PCR amplification of 18S rDNA results

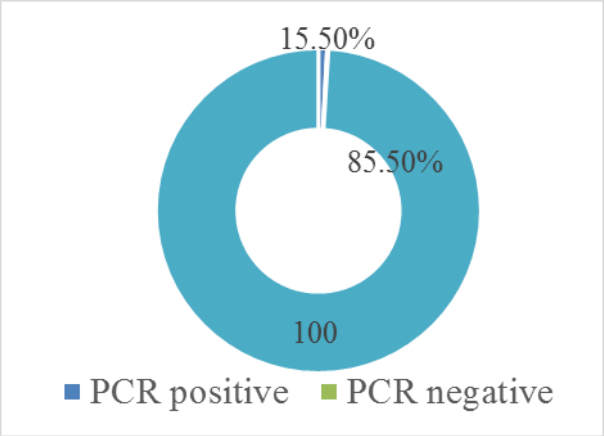

Figure 2. PCR positivity rate of helminthes for18S rDNA among the two breeds of cattle

Table 4.Species of cow intestinal parasites identified by18S rDNA(935bp)sequences(n=60)

Sr.No.	Species of cow intestinal parasites	Number of sequences	Percentage (%)
1.	*Trichostrongylus colubriformis*	26	43.33
2.	*Paroiqolaimella micrura*	15	25.00
3.	*Haemonchus contortus*	5	8.33
4.	*Zoniolaimus mawsonae*	5	8.33
5.	*Fictor stercorarius strain*	3	5.00
6.	*Rhabditis* sp.	3	5.00
7.	*Necator americanus*	1	1.67
8.	*Ancylostoma caninum*	1	1.67
9.	*Panaqrolaimus davidi*	1	1.67
	Total	60	100.00

Table 5. Blast similarity search results of the studied sequences for 18S rDNA in the NCBI GeneBank

ID	query length	ID	Species	Identities	Gaps
CFF2-01	851	AJ920350.1	***Trichostrongylus colubriformis***	843/858(98%)	0/858(0%)
CFF2-02	844	KJ877235	***Fictor stercorarius strain***	818/845(97%)	2/845(0%)
CFF2-03	856	AJ920350.1	***Trichostrongylus colubriformis***	844/856(99%)	0/856(0%)
CFF2-05	853	KJ877235	***Fictor stercorarius strain***	821/849(97%)	2/849(0%)
CFF2-06	859	LS997564.1	***Haemonchus contortus***	839/859(98%)	0/859(0%)
CFF2-07	859	AJ920350.1	***Trichostrongylus colubriformis***	846/859(98%)	0/859(0%)
CMF3-01	853	LS997564.1	***Haemonchus contortus***	833/853(98%)	0/853(0%)
CMF3-02	859	AJ920350.1	***Trichostrongylus colubriformis***	845/859(98%)	0/859(0%)
CMF3-03	843	AJ920350.1	***Trichostrongylus colubriformis***	822/843(98%)	1/843(0%)
CMF3-04	846	AJ920350.1	***Trichostrongylus colubriformis***	832/846(98%)	0/846(0%)
CMF3-05	860	AJ920338.2	***Zoniolaimus mawsonae***	857/860(99%)	1/860(0%)
		AJ920348.1	***Necator americanus***	848/860(99%)	1/860(0%)
		AJ920374.2	***Ancylostoma caninum***	846/860(98%)	1/860(0%)
CMF3-06	846	AJ920350.1	***Trichostrongylus colubriformis***	832/846(98%)	0/846(0%)
CMF3-07	847	AJ920350.1	***Trichostrongylus colubriformis***	833/847(98%)	0/847(0%)
CMF3-08	840	AJ920350.1	***Trichostrongylus colubriformis***	827/840(98%)	0/840(0%)
CMF4-01	846	AJ920350.1	***Trichostrongylus colubriformis***	834/846(99%)	0/846(0%)
CMF4-02	846	AJ920350.1	***Trichostrongylus colubriformis***	832/846(98%)	0/846(0%)
CMF4-03	840	AJ920350.1	***Trichostrongylus colubriformis***	827/840(98%)	0/840(0%)
CMF4-04	859	LS997564.1	***Haemonchus contortus***	842/859(98%)	0/859(0%)
CMF4-05	846	AJ920350.1	***Trichostrongylus colubriformis***	830/846(98%)	0/846(0%)
CMF4-06	853	AJ920350.1	***Trichostrongylus colubriformis***	841/853(99%)	0/853(0%)
CMF4-07	856	AJ920350.1	***Trichostrongylus colubriformis***	841/856(98%)	0/856(0%)
CMF4-08	828	AJ920350.1	***Trichostrongylus colubriformis***	814/826(99%)	1/826(0%)
CMF5-01	847	LS997564.1	***Haemonchus contortus***	833/847(98%)	0/847(0%)
CMM2-01	841	AJ920350.1	***Trichostrongylus colubriformis***	828/841(98%)	0/841(0%)
FF1-01	836	KJ877235.1	***Fictor stercorarius strain***	810/837(97%)	2/837(0%)
FF1-02	807	EU196004.1	***Rhabditis sp.SB347***	778/805(97%)	1/805(0%)
FF1-04	862	EU196004.1	***Rhabditis sp.SB347***	833/865(96%)	5/865(0%)
FF1-05	845	EU196004.1	***Rhabditis sp.SB347***	817/848(96%)	5/848(0%)
FF1-07	837	HQ270131.1	***Panaqrolaimus davidi isolate*AC Pd2**	728/801(91%)	13/801(1%)
FF2-02	841	AJ920350.1	***Trichostrongylus colubriformis***	824/841(98%)	0/841(0%)
FF2-03	839	AJ920338.2	***Zoniolaimus mawsonae***	837/839(99%)	0/839(0%)
FF2-04	829	AJ920338.2	***Zoniolaimus mawsonae***	824/829(99%)	1/829(0%)

FF2-05	840	AJ920350.1	***Trichostrongylus colubriformis***	819/840(98%)	0/840(0%)
FF9-01	870	AJ920338.2	*Zoniolaimus mawsonae*	869/870(99%)	0/870(0%)
MF1-01	890	KJ877207.1	*Paroiqolaimella micrura*	879/891(99%)	1/891(0%)
MF1-02	876	KJ877207.1	*Paroiqolaimella micrura*	865/878(99%)	2/878(0%)
MF1-03	875	KJ877207.1	*Paroiqolaimella micrura*	864/877(99%)	2/877(0%)
MF1-08	878	KJ877207.1	*Paroiqolaimella micrura*	865/878(99%)	2/878(0%)
MF3-01	871	KJ877207.1	*Paroiqolaimella micrura*	862/871(99%)	1/871(0%)
MF3-03	884	KJ877207.1	*Paroiqolaimella micrura*	876/884(99%)	0/884(0%)
MF3-04	878	KJ877207.1	*Paroiqolaimella micrura*	870/879(99%)	1/879(0%)
MF3-05	863	KJ877207.1	*Paroiqolaimella micrura*	855/863(99%)	0/863(0%)
MF3-06	876	KJ877207.1	*Paroiqolaimella micrura*	868/876(99%)	0/876(0%)
MF5-01	839	AJ920350.1	*Trichostrongylus colubriformis*	826/839(98%)	0/839(0%)
MF5-03	825	AJ920350.1	*Trichostrongylus colubriformis*	804/825(97%)	0/825(0%)
MF5-04	841	AJ920350.1	*Trichostrongylus colubriformis*	821/841(98%)	0/841(0%)
MF5-06	846	AJ920350.1	*Trichostrongylus colubriformis*	833/846(98%)	0/846(0%)
MF5-07	846	AJ920338.2	*Zoniolaimus mawsonae*	845/846(99%)	0/846(0%)
MF5-08	840	LS997564.1	*Haemonchus contortus*	822/840(98%)	0/840(0%)
MF8-01	876	KJ877207.1	*Paroiqolaimella micrura*	868/877(99%)	1/877(0%)
MF8-02	790	KJ877207.1	*Paroiqolaimella micrura*	781/790(99%)	0/790(0%)
MF8-03	864	KJ877207.1	*Paroiqolaimella micrura*	856/864(99%)	0/864(0%)
MF8-04	889	KJ877207.1	*Paroiqolaimella micrura*	881/891(99%)	2/891(0%)
MF8-05	864	KJ877207.1	*Paroiqolaimella micrura*	855/864(99%)	0/864(0%)
MF8-06	877	KJ877207.1	*Paroiqolaimella micrura*	869/877(99%)	0/877(0%)
MM3-01	873	AJ920350.1	*Trichostrongylus colubriformis*	860/873(99%)	0/873(0%)
MM3-03	839	AJ920350.1	*Trichostrongylus colubriformis*	819/839(98%)	0/839(0%)
MM3-05	855	AJ920350.1	*Trichostrongylus colubriformis*	843/855(99%)	0/855(0%)

Discussion

Infections with GI nematodes are routinely treated with broad-spectrum anthelmintics, which were derived from only three chemical classes (benzimidazole, levamisole and macrocyclic lactone). Clearly, there is a need to preserve the effectiveness of existing anthelmintics, and this can be supported by more frequent monitoring of worm burdens and adoption of management practices that delay the development of resistance (Leathwick, 2014). The routine diagnosis of GI nematode infections is traditionally based on counting their eggs in a microscopic chamber using a faecal flotation method. (Georgi and McCulloch, 1989) However, this provides little information on the infecting species because apart from *Nematodirus* spp., the eggs of most important GI nematodes are morphologically indistinguishable unless viewed individually at high magnification (Georgi and McCulloch, 1989).It has implications for both the routine monitoring of worm egg counts and testing for anthelmintic resistance using the faecal egg count reduction test (Levecke et al., 2012). Consequently, larval culture (LC) is needed to identify which genera of nematodes are predominant in any given sample. This requires incubation of faecal samples for 7-10 days to hatch infective third-stage larvae (L3), which are then identified using published information

on size and morphology of the important nematode genera and species (Van Wyk et al., 2004). PCR-based methods have key implication to address this question, and several techniques have been developed for the identification and detection of various parasite species (Hu *et al*., 2015). Substantial research in the development of PCR-based methods for the rapid and specific identification GI nematodes has been conducted for small ruminants, whilst only few such assays have been developed for cattle (Roeber et al.,2017). Previous data of the present study shows a total of five nematode species consisting in *Trichuris* sp., *Strongyloides* sp., *Trichostrongylus* sp. *Ascaris* sp. *Nematotida* sp. and two species of common cestodes consisting in *Fasciola hepatica* and *Fasciola magna* had been identified by using both egg morphology and molecular methods. However the combination of morphology and molecular methods yielded more different types of intestinal nematodes that could rarely be detected by microscopy alone. In the present work, molecular identification by PCR and subsequent sequencing of 18Sr DNA marker revealed additional nine species of intestinal nematodes to occur including *Trichostrongylus colubriformis, Paroiqolaimella micrura, Haemonchus contortus, Zoniolaimus mawsonae*, *Fictor stercorarius, Rhabditis* sp., *Necator americanus, Ancylostoma caninum,* and *Panaqrolaimus davidi* among the two breeds of cattle studied in the present study.*Trichostrongylus colubriformis* was found to be the most commonest species(43.3%) detected by molecular method in this study. In this study, *Haemonchus contortus* **(**Trichostrongyloidea) was identified at 8.3% of all intestinal nematodes detected by molecular method. It is a blood-sucking nematode that feeds on blood from capillaries in the abomasum of ruminants, especially for cattle, sheep, and goats. Infections with the nematodes can cause anemia, weight loss, or even death in severely affected animals. Thus, molecular verification of this important species in Myanmar cows is a beneficial information for the control measures. The suborder Strongylida includes many of the important nematodes found in the gastrointestinal tracts of ruminants, horses, and swine, as well as the lungworms of ruminants and the hookworms of dogs and cats. Chabertiidae is one of its families and *Zoniolaimus mawsonae,* is one of its members (World species,2020) and it is newly identified at (8.3%) in the present study. In the present study, larvae were collected from agar culture plates which were used to identify *Rhabditis* sp. to occur at 5% by molecular method among the cattle from Sagaing Region of Myanmar and this is also a new finding in the study area. *Necator americanus* and *Ancylostoma caninum* (intestinal hookworms) also detected in this study area, both at 1.67% each by the molecular method. *Necator americanus* is also known to infect humans. *Ancylostoma caninum* is a normal parasite of dogsMolecular verification of *Necator americanus* and *Ancylostoma caninum* from cow fecal samples. In this study, it may be due to the fact that cows and dogs were raised close to the human dwellings in Myanmar villages and thus there might be cross infections of hookworms from one species to another or through possible zoonotic transmission to human. However, it is noteworthy that the nematode and cestode species, identified by conventional microscopic examination of eggs, could not be found among the species identified by the molecular method.

Conclusion

Molecular identification of cattle intestinal parasites revealed nine additional species to the seven species found by microscopy. The present study highlighted the essential role of molecular techniques in addition to the conventional diagnostic methods for identification down to the species level of the cow intestinal parasites. The new more precise findings from this study will be beneficial for the epidemiology, treatment and control of intestinal parasites in the animal husbandry sector of the economy of our country.

Acknowledgements

I am deeply indebted to Professor Dr.Kay Lwin Tun, Head of Zoology Department, University of Yangon for her kind permission to conduct this research work .I would like to extend my sincere gratitude to Supervisor, Dr. Nyein Nyein Hlaing, Professor and Head ,Department of Zoology, Dagon University for her close supervision and valuable suggestions rendered throughout my research and also wish to acknowledge my Co supervisor Dr Myo Pa Pa Thet Hnin Htwe Aung ,Professor and Head , Department of Microbiology, University of Medicine Taungyi ,for her valuable encouragements. I would like to thank to Dr Myint Oo (Director/Professor (Retd))MOH/MOST, Consultant Clinical Biochemist for his advice. I would like to extend my thank to Eiji Nagayasu Associate Professor,Division of Parasitology,Department of Infectious Diseases, Faculty of Medicine, University of Miyazaki, Japan, for molecular verification.

References

Georgi, J. and McCulloch, C. 1989. Diagnostic morphometry: identification of helminth 685 eggs by discriminant analysis of morphometric data. *Proc. Helm. Soc. Wash*. 56, 44-686 57. 687

Gunathilaka N., Niroshana D., Amarasinghe D. and Udayanga L.2018. Prevalence of Gastrointestinal Parasitic Infections and Assessment of Deworming Program among Cattle and Buffaloes in Gampaha District, Sri Lanka.*BioMed Research International.*Article ID 3048373

Hu W., Wu S., Yu X.,Abullahi A. Y.,Song M,Wang T. Z., Jiang B., and Li G.2015. A Multiplex PCR for Simultaneous Detection of Three Zoonotic Parasites *Ancylostoma ceylanicum*, *A. caninum,* and *Giardia lamblia* Assemblage A.*BioMed Research International*

Leathwick D., 2014. Sustainable control of nematode parasites – a New Zealand 710 perspective. *Small Ruminant Res.* 118: 31-34

Levecke, B., Rinaldi, L., Charlier, J., Maurelli, M.P., Bosco, A., Vercruysse, J., 712 Cringoli, G., 2012. The bias, accuracy and precision of faecal egg count reduction 713 test results in cattle using McMaster, Cornell-Wisconsin and FLOTAC egg counting 714 methods. *Vet. Parasitol*. 188, 194-199

Nongmaithem O, Shantikumar T, Dutta S. 2019. Evaluation of various culture techniques for identification of hookworm species from stool samples of children. *Indian J Med Res* 149 : 539-543.

Nurcahyo R. W., Ekawasti F., Haryuningtyas D., Wardhana A. H., Firdausy L. W., Priyowidodo D. and Prastowo J.2021. Occurrence of gastrointestinal parasites in cattle in Indonesia *Earth and Environmental Science* :686

Roeber, F., Hassan, E. B., Skuce, P., Morrison, A., Claerebout, E., Casaert, S., Homer, D.R., Fireston, S., Stevenson, M., Smith, L. and Larsen, J. 2017. An automated, multiplex tandem PCR platform for the diagnosis of gastrointestinal nematode infections in cattle: An Australian-European validation study. *Vet. Parasitol;* 239:62-75. https://doi.org/10.1016/j.vetpar. 2017.04.011.

van Wyk, J.A., Cabaret, J., Michael, L.M.2004. Morphological identification of 751 nematode larvae of small ruminants and cattle simplified. *Vet. Parasitol.* 119: 277–752