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*Original article*

# MOLECULAR IDENTIFICATION OF ABOMASAL NEMATODES (*HAEMONCHUS SPP*. AND *OSTERTAGIA OSTERTAGI*) IN NATURALLY INFECTED CALVES

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**Summary**

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Clear variation in the pathogenicity and epidemiology of gastrointestinal nematodes and sensitivity to anthelminthic drugs require precise epidemiological information for such worms. Our work is the first effort to detect the abomasal nematodes (*Haemonchus* spp, *Ostertagia ostertagi*) in calves in Mosul, Iraq through amplification of rDNA ITS1 and ITS 2 regions. Four hundred and eighty (480) faecal samples were collected from 35 private calf herds between October 2021 and April 2022. Ages, origins of the herds, size of the herds, and gender were recorded. Microscopic examination and larval culture were conducted. Specified primers were used for molecular identification. The study showed a 50.6% total prevalence of gastrointestinal nematodes in calves. *Haemonchus* spp. and *Ostertagia ostertagi* rates were 62.13%, and 60.9%, respectively. The percentages of L3 of *Haemonchus* spp. and *Ostertagia* spp. were 32% and 14% respectively. Polymerase chain reaction (PCR) showed reaction products of 321 bp, and 257 bp for studied parasites, respectively. In conclusion, classical laboratory tools may be unable to identify precisely the species and subsequently, minimise the comprehension of parasitic species that circulate in animal populations. Molecular detection is a rapid, highly sensitive, specific and cost-effective procedure that accurately identifies parasite species.

**Key words**: *Haemonchus*, *Ostertagia*, PCR, prevalence

## INTRODUCTION

Gastrointestinal parasites affect health, productivity, and profitability of livestock around the world, moreover pose a threat of deworming and drug resistance. Monitoring and control of these parasites is a priority of control programmes, predominantly in tropical and subtropical zones (Maurizio *et al.,* 2021). Infestation with numerous gastrointestinal nematode (GIN) species is widespread in cattle worldwide, with diverse assembly species between countries (Avramenko *et al*., 2017). The majority of digestive worms parasitise their hosts by ingestion of infective larvae or eggs that are already excreted in the faeces from previously affected

animals into the environment that become a source of transmission (Sato *et al*., 2014; Puspitasari *et al*., 2016; Craig, 2018; Income *et al.,* 2021).

Although some GIN like *Haemonchus* spp. and *Ostertagia* spp. are able to induce fatal outcomes, most GIN Infections are subclinical or result in mild disease in the affected animals. Moreover, the severity of parasitic infections may also be assigned to the integrated adverse impact of diverse genera and species and the number of worms, termed parasitic gastroenteritis (Uriarte & Valderrábano, 1989). In addition, the acuteness of infection relies on the immunity and nutritional status of the animal. Usually, invasion of abomasal submucosa by grown worms and penetrating the capillaries using buccal tooth leads to haemorrhage, blood loss, and reduced clotting of blood. About 0.05 mL of blood/day can be lost through ingestion or infiltration from occupied tissue for each single worm, which eventually leads to anaemia and bottle jaw as serum proteins and packed cell volume are decreased (Hildreth & McKenzie, 2020). Substantial losses in the affected herds are related to feeble growth, reduced weight gain, low-grade wool, and mortality of the acutely affected animals (Amarante, 1997; Emery *et al.,* 2016). *Trichostrongylus* sp. infection may clinically display signs such as gain loss, emaciation, inappetence, diarrhoea and even death in intense infection (Getachew *et al.,* 2007).

The genus *Haemonchus* contains about 12 species or more with two major prevailing species: *H. contortus* and *H. placei* (Akkari *et al*., 2013; Hussain *et al*., 2014). Both *H. placei* and *H. contortus* have been documented in large and small ruminants in the field and experimentally proposing the presence of cross-infection and broad range of host species (Hogg *et al.,* 2010; Akkari *et al.,* 2013; Chaudhry *et al.,* 2015). In cattle, *Ostertagia ostertagi* is the commonest pathogenic nematode species (Kim *et al.,* 2015). *Ostertagia* spp. produce serious pathological damage in the abomasum which may clinically result in poor weight, diarrhoea, milk drop and anthelmintic resistance in the affected animals (Craig, 2009; Charlier *et al.,* 2012; Höglund *et al.,* 2013; Peña- Espinoza *et al.,* 2016; Ramos *et al*., 2016).

While conventional procedures are commonly utilised for the diagnosis of gastrointestinal nematodes and anthelmintic resistance in animals, researchers are currently looking for new techniques such as molecular evaluation which are faster, less labour-intensive, and accurate alternatives. Examination of faecal samples is predominantly categorised as either quantitative or qualitative. The quantitative tests identify a specific parasite depending on the characteristic morphology of the parasite's eggs, oocytes, cysts, or larvae (Charlier *et al.,* 2010). However, several limitations are encountered in microscopic examination, like time-consuming and labourous protocols, experience and skills needed for distinction of eggs of analogous appearance and size. Moreover, there are clear gaps between the scientific literature on the length property of diverse nematode parasites and considerable disparity inside the species. Meanwhile, morphometric distinguishing of the larvae varies based on environmental influence including climate (Roeber *et al.* 2013; Roeber & Kahn, 2014; Roeber *et al.,* 2017; Borkowski *et al.,* 2020).

Finally, due to the scarcity of data on the helminths associated with calves in the region, this study was aimed at molecular assessment of distribution of abomasal nematodes of veterinary importance among cattle in Mosul, Iraq.

## MATERIALS AND METHODS

### *Ethical approval*

This research was confirmed by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, the University of Mosul on the 6th of September 2021 (Approval code UM.VET. 2021.20).

### *Animals and location of the study*

Forty hundred eighty (480) calves from thirty-five (35) private herds were included in this study through a cross-sectional survey of different areas in Mosul from October 2021 till the end of April 2022. The animals were: <1 and ≥1 year old, from both sexes, local and imported origins. According to the herd size, they were from small herds (≤40) and large herds (≥40 calves). The epidemiological information mentioned above and the case history were recorded from the breeders, and the most important clinical signs were recorded in a pre-prepared clinical card during farm visiting and sampling process.

### *Collection and handling of faecal samples*

A total of 480 samples of faeces were collected from animals in sterile rubber gloves for each sample, placed in clean, dry, leak-proof, transparent plastic containers, and transported to the laboratory for examination in the clinical pathology laboratory of the Veterinary Medicine College, University of Mosul. Samples that were not examined on the same day were kept cooled at 4 °C to be examined on the following day.

### *Laboratory analysis*

*Faecal egg counts.* For each faecal sample, the egg counting was done by using Mini-FLOTAC technique. In our work this method and according to our knowledge was used for the first time as a new and alternative method to McMaster method. Briefly, two grams of the faecal sample were added to 38 mL of saturated salt solution and homogenised. Then, the two counting chambers in the disc were filled with filtered slurry by the filter on the top cover. After that the disc was left for 10 minutes horizontally. It was transferred to the microscope for examination with a magnification power of 100× (Cringoli *et al.,* 2017; Amadesi *et al.,* 2020). Eggs per gram were calculated using the following equation:

EPG = (total eggs of two chambers) ×10

*Larval culture (LC).* Thirty grams of faeces were laid in a jar and mixed with a little fine charcoal, and drops of water were added to the faeces to maintain the appropriate humidity. The cultures were kept at 25 °C for a period of 1–2 weeks for the purpose of obtaining infective larvae (L3) in order to distinguish them (Roberts & O'Sullivan, 1950; Zajac, 2012; Van Wyk & Mayhew, 2013). Then the larvae were harvested using Baermann technique (Van Wyk & Mayhew, 2013). Moreover, the L3 were collected for identification and additional examinations (Zhou *et al.,* 2019).

*DNA extraction.* The DNA of *Ostertagia spp*. and *Haemonchus spp*. worms was extracted according to the instructions of the manufacturer (FAVORGEN Biotech Corporation Taiwan). Measurement of the extracted DNA was confirmed by agarose gel electrophoresis by mixing 5 µL of the extracted DNA with 1 µL loading dye and placing it in the gel well with the addition of the DNA ladder indicator. The gel was prepared and transported as mentioned later.

*Polymerase chain reaction procedure.* In this study, a 321 bp fragment from the rDNA *ITS-2* region was amplified for

*Haemonchus spp.* using the universal primers NC1 and NC2 proposed by Gasser *et al.* (1993): NC1F- ACGTCTG GTTCAGGGTTGTT and NC2R- TTA GTTTCTTTTCCTCCGCT. The global initiator forward (NC1-F) is located in the *S5.8* subunit gene and the universal initiator reverse (NC2-R) in the *S28* subunit gene. It is known that the *S5.8* gene is located between the *18S* and *S28* genes and is separated from each of them by *ITS1* and *ITS2* respectively. The specific primers for the genus *Ostertagia ostertagi* were HSSF- TAAAAGTCGTAACAAG GTATCTGTAGGT and HSSR- GTCTC AAGCTCAACCATAACCAACCATTG G, 257 bp size at the *ITS1* gene (Zarlenga *et al.,* 2001).

The master mix was prepared with final volume 20 µL. Master mix (10 µL) was mixed with 1 µL (10 pmol/µL) from each of forward and reverse primers, 2 µL of DNA template and 6 µL of PCR grade water. After preparing the PCR tubes, the DNA was amplified in a Thermocycler T 100 TM Thermal Cycler, (Bio-Rad, USA). Thermo-cycling parameters included one cycle of primary denaturation at a temperature of 94 °C for 10 min, 35 cycles (denaturation) at 94 °C for 45 s, annealing at 60 °C for 45 s for *Ostertagia ostertagi* and 55 °C for *Haemonchus* spp., extension at 72°C for 1 min, and final extension at 94 °C for 45 s. At 72 °C for 10 min, the tubes were removed from the device and amplification process was detected on 1.5% agarose gel, power supply 80 V and 300 mA for 60 min. The gel was extracted and placed in a special imaging device Gel Doc EZ Gel Documentation System, (BioRad, USA), then the images were saved for later analysis.

*Statistical analysis*

Total prevalence of gastrointestinal nematodes in calves was calculated in the SPSS program for Windows (v. 21, IBM SPSS, USA).

## RESULTS

Out of 480 faecal samples examined, the proportion of gastrointestinal nematodes in calves was 243/480 (50.6%) depending on the faecal examinations using Mini-FLOTAC methods (Table 1).

In the current study, the prevalence rates of abomasal nematodes *Haemonchus spp*., and *Ostertagia spp*. were 62.13% and 60.9% respectively (Table 2). The results of larval culture indicated that the percentages of L3 of *Haemonchus* spp. and *Ostertagia ostertagi* were 32% and 14% respectively (Table 3).

**Table 1.** The proportion of gastrointestinal nematodes in calves (Mini-FLOTAC method)

| Total number tested | Positive number (%) | Negative number (%) |
|---|---|---|
| 480 | 243 (50.6%) | 237 (49.4%) |

**Table 2.** The proportion of *Haemonchus* spp., and *Ostertagia* spp. among positive animals

| Gastrointestinal nematodes species | Positive animals number (%) |
|---|---|
| *Haemonchus* spp | 151 (62.13%) |
| *Ostertagia ostertagi* | 148 (60.90%) |

**Table 3.** L3 percentage of abomasal nematodes after larval culture

| Type of nematodes | Percentage (%) |
|---|---|
| *Haemonchus* spp | 32% |
| *Ostertagia ostertagi* | 14% |

The results from the PCR showed for the first time the possibility of diagnosing *Haemonchus* spp. in DNA samples in Nineveh Governorate, and to our knowledge in Iraq. They were confirmed on agarose gel using Internal transcribe spacer 2 (*ITS-2*) gene, with reaction product of 321 bp (Fig. 1). The results also showed the possibility of diagnosing *Ostertagia ostertagi* using the specific primer in the form of bundles indicated through the presence of its complement sites at internal transcribe spacer-1 (*ITS-1* region), with a reaction product of 257 bp (Fig. 2).

## DISCUSSION

A high prevalence of gastrointestinal nematodes in calves in Mosul, Iraq, was detected: 50.6%. This result might concur or differ from earlier reports from Iraq

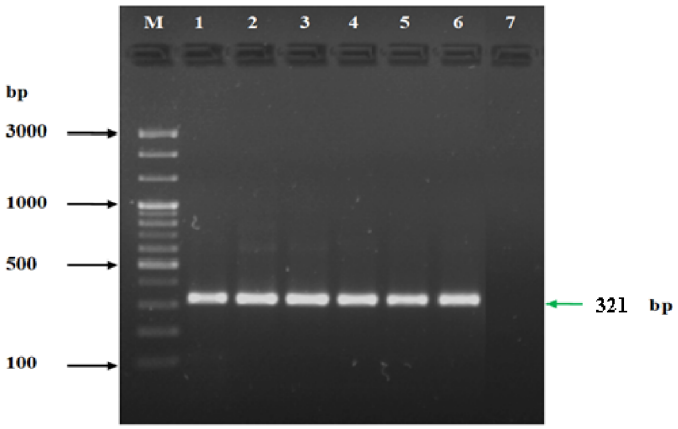


**Fig. 1.** Gel electrophoresis of PCR products for *Haemonchus* spp. M: marker 100 bp of size; lanes 1–6: positive samples (321 bp); lane 7: negative control.

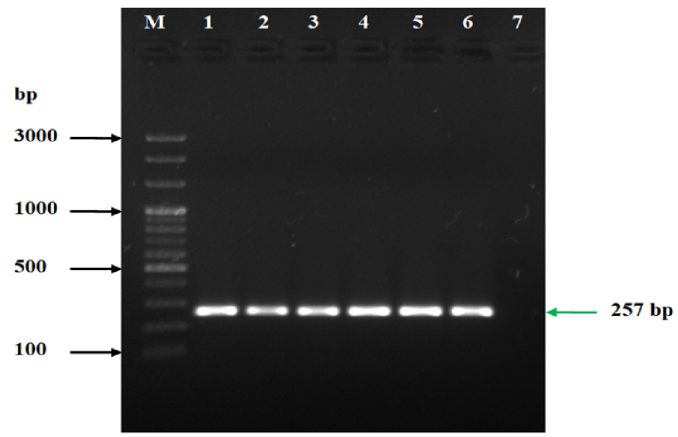


**Fig. 2.** Gel electrophoresis of PCR products for *Ostertagia ostertagi.* M: marker; lanes 1–6: positive samples (257 bp); lane 7: negative control.

and different countries worldwide. Abdulhameed *et al.* (2011) revealed 60.99% total prevalence rate, represented by *Ostertagia* spp., with the highest percentage of 61.62% and *Haemonchus spp* with 40.69%, respectively. A recent study showed 18.60% prevalence of GIT nematodes in Sulaymaniyah (Aram, 2020). The data for Iran showed 81.25% GIT nematodes (Kordi *et al.,* 2019), and in Germany – 41.1% (Gillandt *et al.,* 2018). The reasons for different prevalence rate between the current study and the studies conducted locally and in some countries may be the laboratory tools, management systems, region, climate, sample size, breed and resistance to repellents. The results of this study are in line with those of researchers in different countries worldwide (Rupa & Portugaliza, 2016; Income *et al.*, 2020). It is also to be noted that climatic alteration could affect the recurrence, density, and zone allocation of parasites, which straightly affects the phase of their spread in the surroundings, meanwhile secondarily on the larvae that live mostly in intermediate families of invertebrates. Global warming biologically alters nematodes' distribution range and modifies their development cycles (Okulewicz, 2017; Maurizio *et al.,* 2021).

Our results showed the possibility of diagnosing *Haemonchus* spp. and *Ostertagia* spp. using the specific primer at *ITS-2* gene and *ITS-1* gene, respectively. This finding agrees with the results of previous research (Tan *et al.,* 2014; Elkhatam *et al.,* 2020). It is well known that traditional methods of diagnosing nematodes necessitate experience. At the same time, molecular techniques provide the possibility of additionally effective and authoritative distinction between different types of parasites. It has been pointed out that molecular biological assays are applied to identify and analyse phylogenetically different types of nematodes. This result matches published data (Tan *et al.,* 2014; Sharifdini *et al.,* 2017). It is necessary to point out that the GIT nematodes can be precisely identified through modern methods like molecular techniques because even a single animal could be vulnerable to one or several worm types which can lead to significant impact on health and productivity of the affected animal (Agyei, 1997; Tan *et al.,* 2014).

In summary, in Mosul, classical flotation procedures and microscopic inspection are commonly used in the diagnostic laboratories for the detection of parasite eggs. Though these methods can discriminate parasites, knowledge of genus and species is difficult to be obtained precisely, given the need for additional efforts for inexpert staff. Moreover, the larval culture is also a facility for distinguishing the specific genus properties at larval stage, but unluckily needs practical skills of staff and is time-consuming. Furthermore, differentiation up to species level might be questionable (Vlassoff, 1973; Agyei, 1997).

## CONCLUSION

According to what was mentioned above, the study demonstrated a high prevalence rate of GIT nematodes in calves in the study region with *Haemonchus* spp. and *Ostertagia ostertagi* being the predominant species. The PCR approaches can be utilised as precise complementary tools for identification of worm species to avert the limitations related to morphology-based methods.

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## REFERENCES

Abdulhameed, M. A., Q. T. Al-Obaidy, S. A. Esmaeel & K. J. Hussain, 2011. Clinical and therapeutic study of gastrointestinal parasites in fedlot calves in Goggeli region. *Iraqi Journal of Veterinary Sciences*, **26**, 23–27.

Agyei, A. D., 1997. Seasonal changes in the level of infective strongylate nematode larvae on pasture in the coastal savanna regions of Ghana. *Veterinary Parasitology*, **70**, 175–182.

Akkari, H., J. Jebali, M. Gharbi, M. Mhadhbi, S. Awadi & M. A. Darghouth, 2013. Epidemiological study of sympatric *Haemonchus* species and genetic characterization of *Haemonchus contortus* in domestic ruminants in Tunisia. *Veterinary Parasitology*, **193**, 118–125.

Amadesi, A., A. Bosco, L. Rinaldi, G. Cringoli, E. Claerebout & M. P. Maurelli, 2020. Cattle gastrointestinal nematode egg-spiked faecal samples: High recovery rates using the Mini-FLOTAC technique. *Parasites and Vectors*, **13**, 230.

Amarante, A. F. T., J. Bagnola, M. R. V. Amarante & M. A. Barbosa, 1997. Host specificity of sheep and cattle nematodes in São Paulo state, Brazil. *Veterinary Parasitology*, **73**, 89–104.

Aram, A. M., 2020. Carpological study of gastrointestinal parasites in dairy cattle in Sulaymaniyah province, Kurdistan region, Iraq. *Applied Ecology and Environmental Research,* **18**, 7279–7287.

Avramenko, R. W., E. M. Redman, R. Lewis, M. A. Bichuette, B. M. Palmeira, T. A. Yazwinski, & J. S. Gilleard, 2017. The use of nemabiome metabarcoding to explore gastro-intestinal nematode species diversity and anthelmintic treatment effectiveness in beef calves. *International Journal for Parasitology*, **47**, 893–902.

Borkowski, E. A., E. M. Redman, R. Chant, J. Avula, P. I. Menzies, N. A. Karrow, B. N. Lillie, W. Sears, J. S. Gilleard & A. S. Peregrine, 2020. Comparison of ITS-2 rDNA nemabiome sequencing with morphological identification to quantify gastrointestinal nematode community species composition in small ruminant feces. *Veterinary Parasitology*, **282**, 109104.

Charlier J., J. Demeler, J. Höglund, G. von Samson-Himmelstjerna, P. Dorny & J. Vercruysse, 2010. *Ostertagia ostertagi* in first-season grazing cattle in Belgium, Germany and Sweden: General levels of infection and related management practices. *Veterinary Parasitology*, **171**, 91–98.

Charlier, J., M. Van der Voort, H. Hogeveen & J . Vercruysse, 2012. A novel tool to evaluate the economic importance of worm infections on the dairy farm. *Veterinary Parasitology*, **184**, 204–211.

Chaudhry, U., E. M. Redman, M. Abbas, R. Muthusamy, K. Ashraf & J. S Gilleard, 2015. Genetic evidence for hybridisation between *Haemonchus contortus* and *Haemonchus placei* in natural field populations and its implications for interspecies transmission of anthelmintic resistance. *International Journal for Parasitology*, **45**, 149–159.

Craig, T. M., 2009. Helminth parasites of the ruminant gastrointestinal tract. In: *Food Animal Practice*, 5th edn, Elsevier, pp. 78–91.

Craig, T. M., 2018.Gastrointestinal nematodes, diagnosis and control. *Veterinary Clinics of North America: Food Animal Practice*, **34**, 185–199.

Cringoli, G., M. P. Maurelli, B. Levecke, Antonio Bosco, J. Vercruysse, J. Utzinger & L. Rinaldi , 2017. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. *Nature Protocols*, **12**, 1723–1732.

Elkhatam, A., R. M. AbouLaila, N. Elbahy & M. T. Roshdy, 2020. Microscopical and molecular diagnosis of gastrointestinal nematodes infecting small ruminants in Menofia governorate. *IOP Conference Series: Earth and Environmental Science*, **494** , 012006.

Emery, D. L., P. W. Hunt & L. F. Le Jambre, 2016. *Haemonchus contortus*: The then and now, and where to from here? *International Journal for Parasitology*, **46**, 755–769.

Gasser, R. B., B. N. Chilton, H. Hoste & I. Beveridge, 1993. Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. *Nucleic Acids Research*, **21**, 2525–2526.

Getachew, T., P. Dorchies & P. Jacquiet, 2007. Trends and challenges in the effective and sustainable control of *Haemonchus contortus* infection in sheep. Review. *Parasite,* **14**, 3–14.

Gillandt, K., J. Stracke, T. Hohnholz, R. Waßmuth & N. Kemper, 2018. A field study on the prevalence of and risk factors for endoparasites in beef suckler cow herds in Germany., *Agriculture*, **8**, 1–10.

Hildreth, M. B. & J. B. McKenzie. 2020. Epidemiology and control of gastrointestinal nematodes of cattle in northern climates. *Veterinary Clinics of North America: Food Animal Practice*, **36**, 59–71.

Hogg, R., K. Whitaker, R. Collins, P. Holmes, S. Mitchell, J. Anscombe, L. Redman & J. Gilleard, 2010. Haemonchosis in large ruminants in the UK. *The Veterinary Record,* **166**, 373–374.

Höglund, J., A. Engström, G. von Samson-Himmelstjerna, J. Demeler & E. Tydén, 2013. Real-time PCR detection for quantification of infection levels with *Ostertagia ostertagi* and *Cooperia oncophora* in cattle faeces. *Veterinary Parasitology,* **197**, 251–257.

Hussain, T., K. Periasamy, A. Nadeem, M. B. Ellahi, R. Pichler & A. Diallo, 2014. Sympatric species distribution, genetic diversity and population structure of *Haemonchus* isolates from domestic ruminants in Pakistan. *Veterinary Parasitology,* **206**, 188–199.

Income, N., J. Tongshoob, S. Taksinoros, P. Adisakwattana, C. Rotejanaprasert, P. Maneekan & N. Kosoltanapiwat, 2021. Helminth infections in cattle and goats in Kanchanaburi, Thailand, with focus on strongyle nematode infections. *Veterinary Sciences,* **8**, 324.

Kim, E. S., T. S. Sonstegard, M. V. G. B. Da Silva, L. C. Gasbarre & C. P. Van Tassell, 2015. Genome-wide scan of gastrointestinal nematode resistance in closed Angus population selected for minimized influence of MHC. *PLoS ONE,* **10**, e0119380.

Kordi, B., M. Mirzaei & M. Nooshadokht, 2019. Identification of abomasum nematodes fauna of ruminants in Kerman industrial slaughterhouse, Iran. *Biomedical Journal of Scientific & Technical Research,* **20**, 14970–14973.

Maurizio, A., A. F. di Regalbono & R. Cassini, 2021. Quantitative monitoring of selected groups of parasites in domestic ruminants: A comparative review. *Pathogens,* **10**, 1173.

Okulewicz, A., 2017. The impact of global climate change on the spread of parasitic nematodes. *Annals of Parasitology*, **63**, 15–20.

Puspitasari, S., A. Farajallah & E. Sulistiawati, 2016. Effectiveness of ivermectin and albendazole against *Haemonchus contortus* in sheep in West Java, Indonesia. *Tropical Life Sciences Research,* **27**, 135–144.

Roberts, F. & P. J. O'Sullivan, 1950. Methods for egg counts and larval cultures for strongyles infesting the gastro-intestinal tract of cattle. *Australian Journal of Agricultural Research,* **1**, 99–102.

Roeber, F. & L. Kahn, 2014. The specific diagnosis of gastrointestinal nematode infections in livestock: Larval culture technique, its limitations and alternative DNA-based approaches. *Veterinary Parasitology,* **205**, 619–628.

Roeber, F., A. R. Jex & R. B. Gasser, 2013. Next-generation molecular-diagnostic tools for gastrointestinal nematodes of livestock, with an emphasis on small ruminants: A turning point? *Advances in Parasitology,* **83**, 267–333.

Roeber, F., E. B. Hassan, P. Skuce, A. Morrison, E. Claerebout, S. Casaert, D. R. Homer, S. Firestone, M. Stevenson, L.

Smith & J. Larsen, 2017. An automated, multiplex-tandem PCR platform for the diagnosis of gastrointestinal nematode infections in cattle: An Australian-European validation study. *Veterinary Parasitology,* **239**, 62–75.

Rupa, A. P. M. & H. P. Portugaliza, 2016. Prevalence and risk factors associated with gastrointestinal nematode infection in goats raised in Baybay city, Leyte, Philippines. *Veterinary World*, **9**, 728–734.

Sato, M. O., M. Sato, W. K. Chaisiri, T. Maipanich, S. Yoonuan, T. Sanguankiat, B. Pongvongsa, M. K. Boupha & J. Waikagul, 2014. Nematode infection among ruminants in monsoon climate (Ban-Lahanam, Lao PDR) and its role as food-borne zoonosis. *Revista Brasileira de Parasitologia Veterinária,* **23**, 80–84.

Sharifdini, M., Z. Heidari, Z. Hesari, S. Vatandoost & E. Beigom , 2017. Molecular phylogenetics of *Trichostrongylus* species (Nematoda: Trichostrongylidae) from humans of Mazandaran Province, Iran. *Korean Journal of Parasitology,* **55**, 279–285.

Tan, T. K., C. Panchadcharam, V. L. Low, S. C. Lee, R. Ngui, R. S. K Sharma & Y. A. Lim, 2014. Co-infection of *Haemonchus contortus* and *Trichostrongylus* spp. among livestock in Malaysia as revealed by amplification and sequencing of the internal transcribed spacer II DNA region. *BMC Veterinary Research*, **10**, 38.

Uriarte, J. & J. Valderrábano, 1989. An epidemiological study of parasitic gastroenteritis in sheep under an intensive grazing system. *Veterinary Parasitology,* **31**, 71–81.

VanWyk, J. A. & E. Mayhew, 2013. Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. *Onderstepoort Journal of Veterinary Research,* **80**, 539.

Vlassoff, A., 1973. Seasonal incidence of infective trichostrongyle larvae on pasture grazed by lambs. *New Zealand Journal of Experimental Agriculture*, **1**, 293–301.

Zajac, A. M. & G. Conboy, 2012. Veterinary Clinical Parasitology, 8th edn, Wiley-Blackwell, West Sussex, UK.

Zarlenga, D. S., M. B. Chute, L. C. Gasbarre & P. C. Boyd, 2001. A multiplex PCR assay for differentiating economically important gastrointestinal nematodes of cattle. *Veterinary Parasitology,* **97**, 199–209.

Zhou, S., D. Harbecke, & A. Streit, 2019. From the feces to the genome: A guideline for the isolation and preservation of *Strongyloides stercoralis* in the field for genetic and genomic analysis of individual worms. *Parasites and Vectors,* **12**, 496.

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