Iraqi Journal of Veterinary Sciences

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Traditional and molecular identification of *Haemonchus contortus* and Eimeria spp in slaughtered sheep in Al-Diwaniyah city, Iraq

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Article information

Article history:
Received 03 August, 2023
Accepted 27 October, 2023
Published online 31 January, 2024

Keywords:
Eimeria spp
Haemonchus contortus
Enteroparasites
Sheep parasites

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Abstract

Ovine haemonchosis always a possible cause of anemia or mortality in sheep, and its need much moisture to survive and is rare in dry parts of the globe. The economic important of coccidiosis of one or more species of Eimeria that cause infections in sheep, its widely thought to infect sheep until the last three decades. These parasites leading causes of anemia in sheep in several world regions. The current study was conducted to traditionally and molecularly detect *Haemonchus contortus* and *Eimeria* spp in intestinal fecal contents of slaughtered sheep in Al-Diwaniyah city, Iraq. The investigation started with collecting 170 samples of fecal contents from intestines of slaughtered sheep. The samples were exposed to microscopic examination (flotation method) and real-time quantitative PCR (RT-qPCR). The findings of the microscopic examination revealed the presence of *H. contortus* eggs in 63 (37.1%) samples and *Eimeria* spp oocysts in 39 (22.9%) samples of the intestinal contents. The RT-qPCR showed that *H. contortus* was detected in 49/63 (77.8%) samples and *Eimeria* spp in 18/39 (46.2%) positive microscopic samples of the intestinal contents. The present investigation displays significant *Haemonchus contortus* and *Eimeria* spp occurrences in the intestinal content samples of the examined sheep from Al-Diwaniyah city, Iraq.

DOI: 10.33899/ijvs.2023.142307.3168,

Introduction

Sheep anemia may be caused by several different conditions. One such condition is haemonchosis, which is caused by *Haemonchus contortus*. It is one of the leading causes of anemia in small ruminants in several world regions (1-6). The parasitic nematode *H. contortus* belongs to the *Trichonstrongylidae* family of the *Strongylida* order of the Nematoda phylum. *H. contortus* is well-suited to many climates, although it does exceptionally well in rainfall tropical regions (7-9). Historically, haemonchosis was more prevalent in high-risk places, but as the globe warms and new regions become habitable, *H. contortus* may be able to live and thrive there as well. Therefore, haemonchosis should always be considered a possible cause of anemia or mortality in sheep (10-13). *H. contortus* requires a warm, wet environment for its free-living stages to thrive and persist outside the host. Haemonchosis may happen at any time of year if the right circumstances occur. Disease is most common, and *H. contortus* is most likely to survive in regions with a tropical environment (14,15). Larvae can survive and develop in the humid summers and autumns in the warm temperate. Because the free-living larval stages of *H. contortus* need much moisture to survive, haemonchosis is rare in dry parts of the globe. Larvae may live in warmer, dry locations with enough precipitation or irrigation (16-18). Temperatures between 22 and 26°C and relative humidities around 100% are ideal for the hatching and developing *H. contortus* eggs and larvae. Larvae may overwinter in parched feces and contribute to an outbreak following rainfall. The larval development period is more variable but may be as little as four days under optimal circumstances (from egg to

infective third-stage larvae). Low humidity and desiccation kill eggs and larvae quickly (19-21). The unsheathed L3 larvae, which are the most robust free-living form and may live for extended periods provided the temperature and humidity are favorable, retain the cuticle of the second-stage larvae. The L3 emerges from the feces, lands on the soil, and proceeds laterally and vertically without needing supplemental water (22). The larvae do not eat and instead depend on their reserves. Larvae may survive the winter in various areas because they are dormant (more than freezing), need little energy, and can live for an extended time. Since L3s are more active and utilize energy reserves faster in tropical conditions, their average lifespan is fewer than five weeks (23,24). The economic impact of ovine coccidiosis has been highly documented. One or more species of Eimeria cause infections in sheep, with *E. ovinoidalis* and *E. crandallis* being the most dangerous. Eimeria spp. was widely thought to infect sheep and goats until the last three decades. Trials of interspecies transmission showed that Eimeria is species-specific in sheep and goats (25).

The current study was conducted to traditionally and molecularly detect *Haemonchus contortus* and *Eimeria* spp in intestinal fecal contents of slaughtered sheep in Al-Diwaniyah City, Iraq.

Materials and methods

Ethical approve

The study was approved and carried out at the College of Veterinary Medicine, University of Al-Qadisiyah with approval number (P.G, No. 1890 in 2020) during the period September to December 2022 according to the international guidelines for the care and use of animals.

Samples

The investigation started with collecting 170 samples of fecal contents from the intestines of slaughtered sheep. The study was conducted from September to December 2022. The intestinal content samples were taken and transported in a cool box to the Laboratory of Parasitology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq.

Microscopic examination

The flotation technique was followed to separate the oocysts of the parasites. Standard fecal analysis calls for centrifuging 2-5 g of feces and weighing the resulting pellets. 10 ml of the saturated salt solution should be added to the debris. The contents were poured through a fine mesh strainer into a beaker. The filtered mixture has to be placed in a 15-ml centrifuge tube. The mix was left to make a thin layer of flotation solution to the tube. The centrifuge was set at 1,200 RPM (280 g) for 5 minutes. The method was previously performed by Dryden *et al.* (26).

Extraction of DNA

The DNA extraction was done using methods from Högberg *et al.* (27), in which 10ml NaCl-saturated solution was applied to 5gms of intestinal contents. This mixture was gauze-filtered and 4000rpm-centrifuged for 3mins. The supernatant at 500µl was used to start the extraction method. The DNA was evaluated for integrity using a NanoDrop.

Real-time quantitative PCR

The primers purchased from Macrogen (Korea) were F: GGCGGGAACAAGTTGAACAGT and R: CCCTAAAATTGATCTTAAACCC (mt-COI region) for *H. contortus* (28) and F: CGCGCAAATTACCCAATGAA and R: ATGCCCCCAACTGTCCCTAT (18s rRNA gene) for *Eimeria* spp (29). The reaction contained 2µl DNA, 4µl) of H2O2, 10µl water, and 2µl of each primer for a total volume of 20µl. The methods were followed by Zhu *et al.* (30). The thermocycling steps were 40 cycles of denaturation 95ºC, annealing 60-50ºC, and extension 72ºC. The annealing temperatures were different for either parasite (31,32).

Statistical analysis

T-test was used via GraphPrism v7.0 (GraphPad Inc., USA) to compare the levels of both parasites. A p-value of less than 5% was used.

Results

The findings of the microscopic examination revealed the presence of *H. contortus* eggs in 63/170 (37.1%) samples and *Eimeria* spp oocysts in 39/170 (22.9%) samples of the intestinal contents. The RT-qPCR showed that *H. contortus* was detected in 49/63 (77.8%) samples and *Eimeria* spp in 18/39 (46.2%) positive microscopic samples of the intestinal contents (Figure 1).

Discussion

The present study found that *H. contortus* oocysts were identified using microscopy in 37.1% samples. Höglund *et al.* (33) showed that oocysts were found in 56% microscopic samples. The current work identified the occurrence of *Eimeria* spp oocysts in 22.9% microscopic samples. Majeed *et al.* (34) found that microscopic examination recorded the presence of oocysts in 100% fecal samples. Some novel methods for identifying *Haemonchus* spp. appeared with the introduction of RT-PCR. Although RT-PCR was initially developed to measure gene transcription, many labs have begun using it to replace traditional PCR in the identification methods of intestinal parasites (35-37).

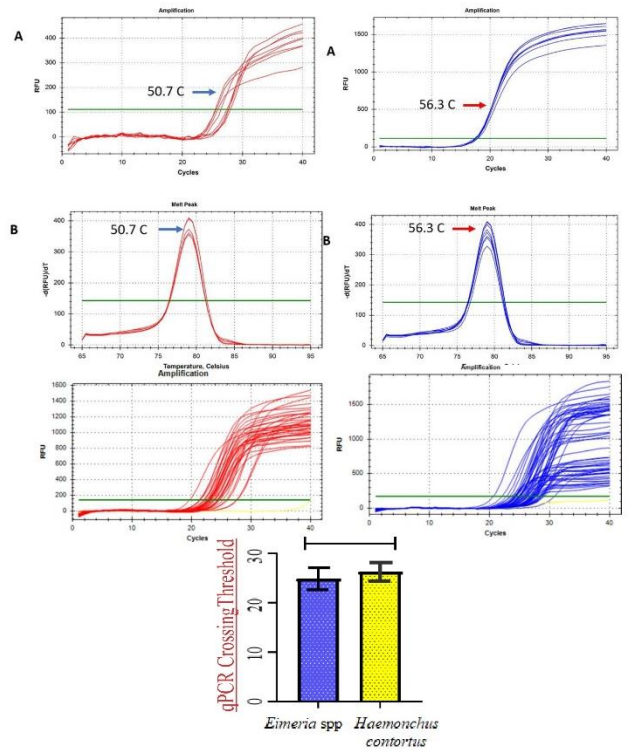

Figure 1: Real-time quantitative PCR of *Eimeria* spp and *Haemonchus contortus* in feces of sheep intestinal content. A and B: Optimization and RT-qPCR findings for *Eimeria* spp (Red) and *Haemonchus contortus* (Blue).

With the advent of real-time PCR, researchers' attention has shifted from assay creation to practical applications. Despite the study's narrow emphasis on cultured larvae, Siedek *et al.* (37) found a strong association between probe-based real-time PCR results and coproculture. The approach of growing to L3 and then examining the parasites morphologically has been thoroughly reviewed (38-40), but since then, researchers have shifted their focus to antemortem PCR-based detection of fecal eggs. Numerous publications have been published in combination with carrying out molecular assays on fecal eggs, in which the separation of eggs was not preceded by purification and DNA isolation. For example, Sweeny *et al.* (41) extracted DNA from ovine feces and successfully performed specific PCR. Egg flotation experiments where the epg was more than 50 correlated well with the data. However, the egg detection borderlines were never identified, and cultures of the fecal eggs were never undertaken to verify the PCR results. To use real-time PCR for quantitative (qPCR) assessment of larval loads on pasture (42). Due to the presence of both L3s and eggs on pasture, a weak relationship was seen between Cts and pasture larval counts. The qPCR results were positive, nevertheless. Subsequently, McNally *et al.* (43) devised a technique for extracting DNA from sheep feces to quantify eggs from *Haemonchus*. Using this method, the test demonstrated 10epg-based sensitivity (42,43).

In mixed-species infections, efforts have been undertaken to quantify fecal eggs. Quantification can determine the densities of eggs. The DNA content and alterations in the gen amplification across species may render molecular amplification of fecal eggs ineffective (44). The first does not seem to be a problem based on anecdotal information, and the latter may be handled by carefully regulating test conditions and parameters. DNA can change its amount, which may interfere with the quantification in the first 6-7 hours following embryonation (45). RT-qPCR was initially used to quantify sheep gastrointestinal nematodes (46-52).

Conclusion

The present investigation displays significant *Haemonchus contortus* and *Eimeria* spp occurrences in the intestinal content samples of the examined sheep from Al-Diwaniyah city, Iraq.

Acknowledgments

The authors thank Professor Jabbar Ahmed Alssady, Dean of the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq, for technical assistance.

Conflict of interests

The authors have not received any funding or benefits from industry, financing agencies, or elsewhere to conduct this study.

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التحديد التقليدي والجزيئي لطفيليات المنقوسات الضأنية والمكيسات البوغية في محتويات البراز المعوي للأغنام المذبوحة في مدينة الديوانية، العراق

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الخلاصة

يعد داء المنقوسات دائما سببا محتملا لفقر الدم أو الوفيات في الأغنام، ويحتاج إلى الكثير من الرطوبة للبقاء على قيد الحياة وهو نادر في الأجزاء الجافة من العالم .الأهمية الاقتصادية لمرض الكوكسيديا لواحد أو أكثر من أنواع الأيميريا التي تسبب العدوى في الأغنام، ويعتقد على نطاق واسع أنها تصيب الأغنام حتى العقود الثلاثة الماضية .وتؤدي هذه الطفيليات إلى الإصابة بفقر الدم لدى الأغنام في العديد من مناطق العالم. أجريت الدراسة الحالية للكشف تقليديا وجزيئيا عن المنقوسات في الضأن والمكيسات البوغية في محتويات براز الأمعاء للأغنام المذبوحة في مدينة الديوانية، العراق. بدأت الدراسة بجمع ١٧٠ عينة من البراز من أمعاء الضأن المذبوحة. تم إجراء الفحص المجهري للعينات (الطريقة العائمة) وفحص أنزيم البلمرة المتسلسل الكمي في الوقت الحقيقي. إذ كشفت نتائج الفحص المجهري عن وجود بيض المنقوسات في الضأن في ٦٣ (٣٧,١٪) عينة وأكياس بيض المكيسات البوغية في ٣٩ (٢٢,٩٪) عينة من محتويات الأمعاء. أظهر فحص إنزيم البلمرة المتسلسل الكمي في الوقت الحقيقي أنه تم الكشف عن المنقوسات في الضأن في ٦٣/٤٩ (٧٧,٨٪) والمكيسات البوغية في ٣٩/١٨ (٤٦,٢٪) من العينات المجهرية الموجبة المأخوذة من محتويات الأمعاء. أظهر البحث الحالي تواجد المنقوسات في الضأن والمكيسات البوغية في عينات المحتوى المعوي للأغنام التي تم فحصها من مدينة الديوانية، العراق.

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