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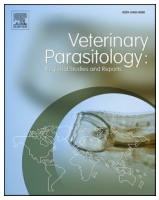

Short Communication

First report of gastrointestinal nematodes and coccidia parasites from free-range chickens in Mafeteng district, Lesotho

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ABSTRACT

Free-range chickens are an integral part of poultry production in many developing countries. In the Mountain Kingdom of Lesotho, the majority of the population own free-range chickens, which serve a variety of purposes including being a source of meat, eggs and use for cultural rituals amongst others. However, there is lack of scientific studies on occurrence of parasitic infections on free-range chickens in Lesotho. The aim of this study was to document common gastrointestinal parasites infecting free-range chickens in four villages of Mafeteng District in Lesotho. A total number of 462 pooled faecal samples were collected from various households in HaKubutu ($n = 114$), HaMatjeka ($n = 120$), HaMpalipali ($n = 120$) and Thabang Villages ($n = 108$) which were subjected to microscopic examination using McMaster technique. The prevalence of gastrointestinal parasite infection was as follows: *Eimeria tenella* (12.8%), *Ascaridia galli* (10.4%) and *Heterakis gallinarum* (5%). The prevalence for *H. gallinarum* and *Ascaridia galli* were comparatively higher during the hot-wet season (7.1% and 2.8% respectively) than the cold-dry season (3.2% and 1.9% respectively) and varied significantly ($P < 0.05$). For *E. tenella*, the oocysts per gram were slightly higher in the cold-dry season than the hot-wet season. Polymerase chain reaction only amplified DNA from six (29%) adult *A. galli* worms of which two amplicons were successfully sequenced. The obtained cytochrome C oxidase subunit 1 partial gene sequences displayed 98–100% identity with South African *A. galli* isolates. This is the first scientific study on prevalence and molecular characterization of nematodes and coccidia species infecting free-range village chickens in Lesotho. The findings can be used to review management of gastrointestinal nematodes and protozoal parasites of free-range chickens in Lesotho.

1. Introduction

Free-range chickens are native chicken breeds (*Gallus* genus) and mostly raised in resource-limited rural villages of developing countries due to their adaptation to harsh environmental conditions and poor farming practices (Elson, 2011; Padhi, 2016). The chickens are mostly fed on leftover food and occasionally fed with specifically formulated chicken ration (Dessie and Ogle, 2001). These chickens scavenge during daytime and are confined in shelters made from locally available material during night-time (Muchadeyi et al., 2009; Mtileni et al., 2009). The current phenotypic and genetic distributions of the breeds were influenced by factors that include previous human migration, mutation due to environmental conditions, artificial and natural selection (Clutton-Brock, 1992). In Lesotho, free-range chickens constitute 48% of the chicken population and survive in all agro-ecological zones (Malibeng, 2018). Currently, there are no breed qualifications for these chickens. Nonetheless, these chickens can be categorized into three groups: Brown and Black Red; Sole Black; and the Dark, Red, Cuckoo, Silver Grey and Spangled birds according to distinct differences in colour of plumage (Nthimo, 2004; Nthimo et al., 2004; Malibeng, 2018).

Gastrointestinal parasite infections in poultry either by helminths or protozoa results in suppression of the immune system, delayed growth, low egg production and sometimes death (Dube et al., 2010; Katoch et al., 2012). Seasons with relatively higher moisture and warm temperature favour oocyst sporulation and hatching of ova and are associated with high parasite occurrences (Kaboudi et al., 2016). The infective

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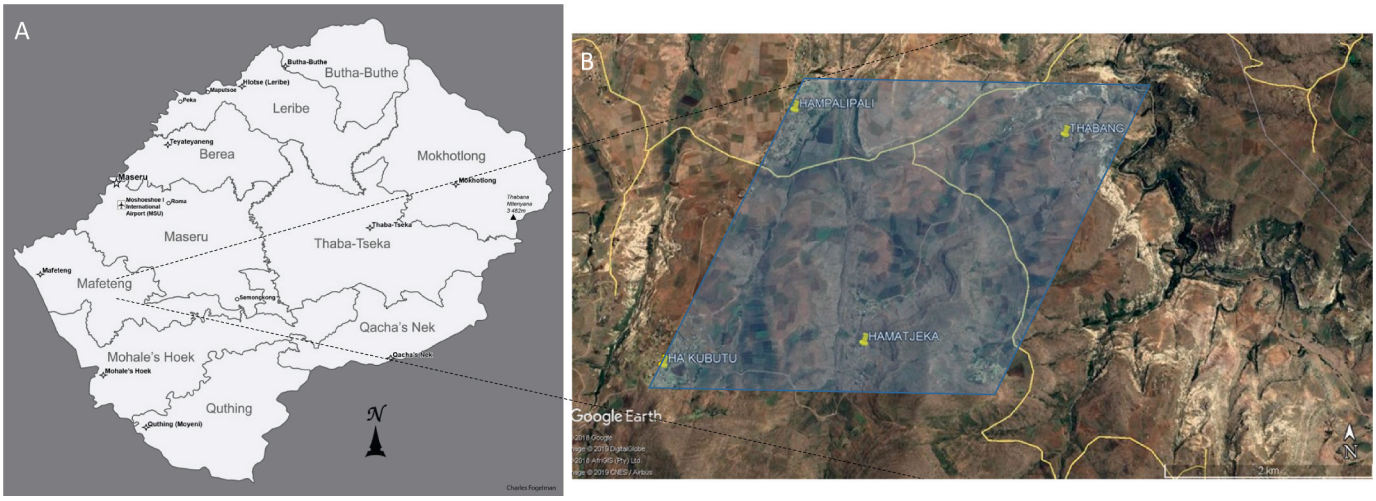

Fig. 1. Sampled area in Mafeteng, Lesotho. (A) The geographical map of Lesotho with its ten districts including Mafeteng district where samples were collected (Wikipedia). (B) Spatial distribution of study villages within Mafeteng district (Google earth).

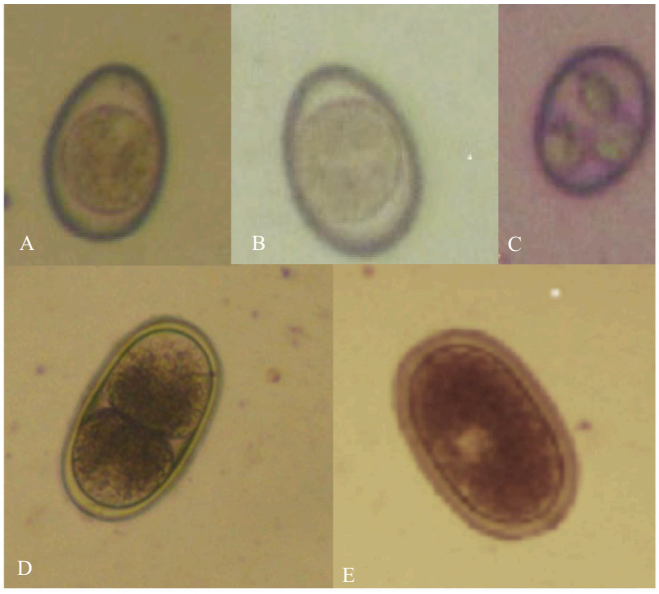

Fig. 2. Photomicrographs of detected *Eimeria* spp. and *Ascaridia* spp. oocysts. (A) Unsporulated *Eimeria* oocyst; (B) Sporulating *Eimeria* oocyst and; (C) Sporulated *Eimeria* oocyst; (D) Embryonating *A. galli* egg with two blastomeres; (E) Mature *A. galli* unembryonated egg.

stages of coccidia and helminths occur in higher numbers in the environment and eventually infect the chickens (Wuthijaree et al., 2019). Poultry that are kept under intensive production system are less exposed to parasitic diseases largely due to housing that excludes parasite as well as improved management practices whereas in free-range birds, the prevalence is high, implying higher exposure to parasitic infections (Abdelqader et al., 2007; Sharma et al., 2013; Sarba et al., 2019).

Nematodes and coccidia are amongst the most important parasites in chickens causing weight decrease, damage to intestinal walls, reduced egg production and increased mortality (Ayeh-Kumi et al., 2016; Belete et al., 2016). Major nematode genera that infect poultry include *Ascaridia, Heterakis* and *Capillaria* (Sarba et al., 2019; Wuthijaree et al., 2019). The most prevalent species include *A. galli, H. gallinarum* and a number of species under the genus *Capillaria* include *C. anatis*, *C. obsignata*, *C. bursata* and *C. caudinflata* (Asumang et al., 2019; Ara et al., 2021). The *Eimeria* species that cause coccidiosis in chicken include *E. acervulina, E. necatrix, E. praecox, E. mitis, E. brunetti, E. maxima* and *E. tenella* (Mcdougald, 1998). The latter is the most prevalent species that is associated with lesions in the caecum of chickens (Huang et al., 2017; Hamid et al., 2018).

Helminths and coccidia are global concerns due to their effects on health, production and welfare of chickens (Shifaw et al., 2021). There is no specific strategy on management of chicken parasites in Lesotho. The farmers control parasites with traditional agents and homemade concoctions such as aloe extracts, potassium permanganate, Jeyes fluid and rarely anthelmintic drugs (Malibeng, 2018). Sub-Saharan countries that include South Africa, Botswana and Zimbabwe conducted studies on gastrointestinal parasites of backyard chickens to improve the understanding on their epidemiology, pathogenesis and management (Thekisoe et al., 2003; Mtileni et al., 2009; Mwale and Masika, 2011). The information is particularly important for rural populations in developing countries where extensive chicken farming is one of the main sources of protein and income (Dube et al., 2010; Desta, 2021). Currently, there is a paucity of information on the prevalence, faecal egg count, and the role of seasons on the variation of parasites of free-range chickens in Lesotho. Therefore, this study was undertaken to document the occurrence of gastrointestinal parasites infecting free-range chickens in Mafeteng District of Lesotho.

2. Materials and methods

2.1. Study area and sample collection

The study was conducted in Mafeteng District that is located on the southern part of Lesotho, bordering with Maseru district, Mohale's Hoek District and the Free State Province in South Africa. The samples were collected on a monthly basis for a period of 12 months from 39 households that had a minimum of 15 village/backyard chickens that were free ranging at HaKubutu [29°44′16.7"S 27°35′51.0″E], HaMatjeka [29°43′54.8"S 27°37′24.7″E]), HaMpalipali [29°45′16.5"S 27°35′25.9″E] and Thabang [29°45′09.4"S 27°36′26.4″E] villages (Fig. 1). The duration was divided into wet-hot (ranging from October to March) and dry-cold seasons (ranging from April to September) based on differences in temperature and rainfall (Grundling et al., 2015). The top parts of freshly voided faecal samples were collected in pools early in the morning from each household into separate sterile capped bottles bearing the details of the household, date of collection and location. The samples were immediately transported to the laboratory in a cooler box with ice packs, stored at 4 °C and processed within two weeks.

Six chickens which were found dead during sample collection were

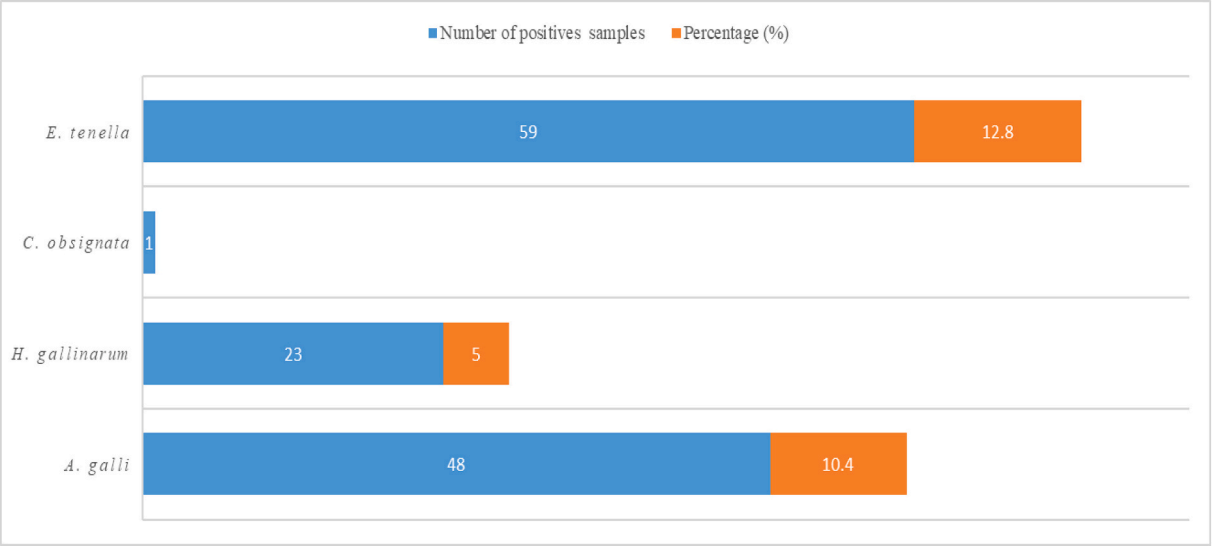

Fig. 3. Number of positive samples with percentages for all detected parasites species.

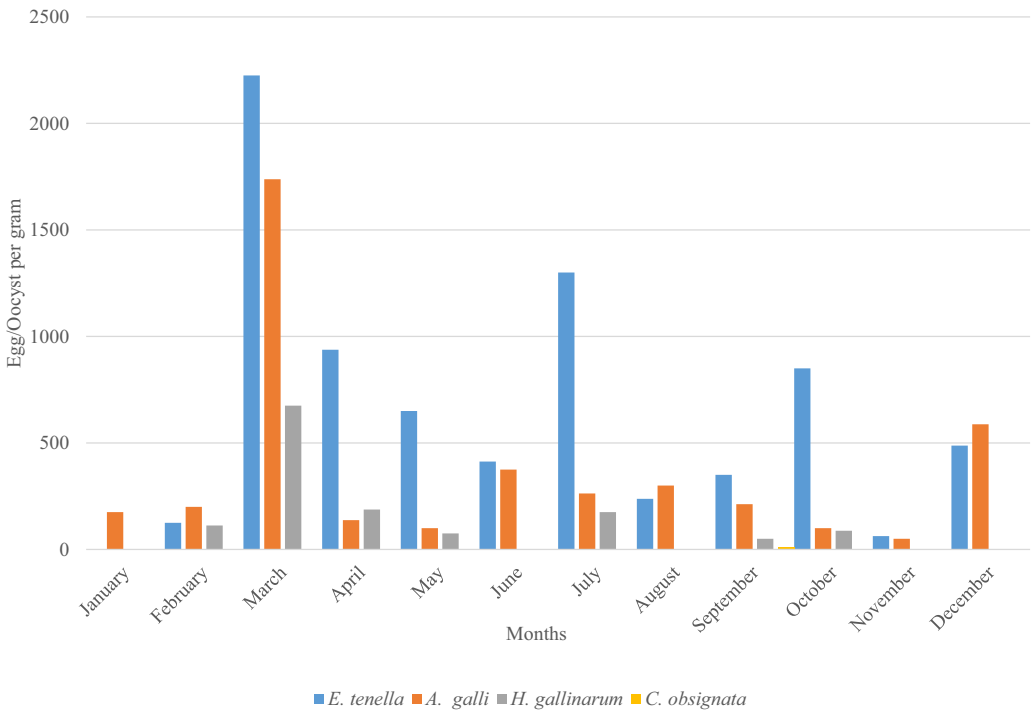

Fig. 4. Monthly prevalence of nematode eggs based on combined overall EPG/OPG counts in four villages of Mafeteng.

taken for post morterm examination at the Central Veterinary Laboratory in Maseru. During the postmortem, complete gatsrointestinal tracts were collected, cut into smaller sections and opened longitutinally. Digesta, mucosal scrapings and washes were observed for presence of parasites for each section (Khan et al., 2016). All visible adult worms and a pool of unidentified growth-stages of parasites were collected and stored in respective tubes with 70% ethanol until identification and extraction of DNA.

2.2. Laboratory examination

Pooled faecal samples from each household were homogenised in a blender with saturated salt solution and examined using a simple flotation technique following previoulsy described guidelines Permin and Hansen (1998). The helminth eggs and coccidian oocysts were identified using the published key guide of Soulsby (1982). Because of the similarities between *A. galli* and and *H. gallinarum* eggs, the following charecteristics were used to differentiate between the two parasites' eggs: eggs with thick-shells and elliptically shaped and measured 65–80 by 35–46 μm were considered as positive identification of *H. gallinarum,* while eggs with smooth shells, oval shaped and measured 73–92 by 45–57 μm were considered as *A. galli*. The identies of the observed eggs and oocysts were further confirmed by reference to pictures from the published key. After identification, the eggs and oocysts were quantified

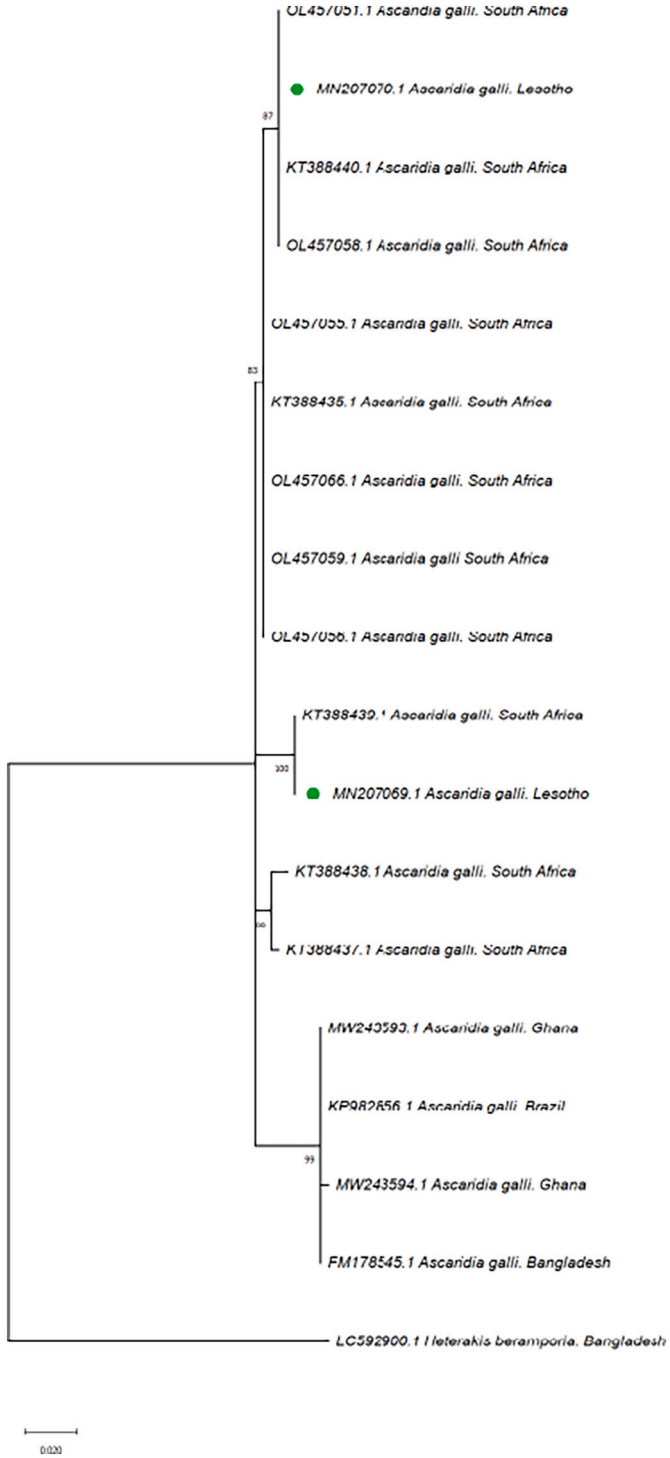

Fig. 5. Phylogenetic tree of *A. galli* based on COX1 gene using Neighbour Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10,000 replicates) are shown next to the branches. The two clades in green boxes show *A. galli* Lesotho isolates sharing clusters with South African isolates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

using a McMaster technique under light microscope (Olympus, Japan) (Soulsby, 1982; Permin and Hansen, 1998). The eggs/oocysts per gram of faeces (EPG/ OPG) were determined for positive samples following the protocol described by Permin and Hansen (1998). The adult worms (21) were morphologically identified under the microsocope using keys described by Soulsby (1982).

Six adult worms and pooled different growth-stages of helminths were crushed with pestle and motor and mixed with phosphate buffered saline (PBS). The helminth DNA was extracted using a QIAamp DNA extraction kit according to manufacturer's instructions (Qiagen, USA) followed by determination of concentration with a NanoDrop 200/200c Spectrophotometer (Thermo Scientific, USA). PCR primers were used to amplify chicken gastrointestinal parasite DNA as described in published literature (Schnitzler et al., 1998; Floyd et al., 2005; Katakam et al., 2010; Tamaru et al., 2015; Gu et al., 2016). The positive PCR products were sent for Sanger sequencing at Inqaba Biotechnical Industries (Pty, Ltd.) in South Africa.

The resultant DNA sequences were edited using BioEdit Sequence Editor Software version 7.2.5 (Bioedit Company) and identified using the nucleotide Basic Local Alignment Search Tool (BLASTn) (http://blast.ncbi.nlm.nih.gov/). Furthermore, the *A. galli* cytochrome C oxidase subunit 1 (COX1) partial gene sequences generated in this study were deposited into the National Center for Biotechnology Information (NCBI) GenBank™ database under accession numbers: **MN207069** and **MN207070**. For the phylogenetic analysis, comparative sequences of *A. galli* were downloaded from GenBank and aligned with the sequences generated in this study using ClustalW alignment tool implemented in MEGA X. The alignment consisted of 18 sequences and was 367 nucleotides long. The evolutionary history was inferred by using the Maximum Likelihood (ML) method on MEGA X software (Kumar et al., 2018).

2.3. Statistical analysis

All data analyses were conducted in Microsoft Excel 2013. Non-parametric one-way Analysis of Variance (ANOVA), with confidence level at 95%, was calculated to determine the significance of difference in the detected parasites amongst the four villages, over the period of 12 months as well as between the detected parasites. Furthermore, Chi-squared test of independence at 95% confidence level, was conducted to determine the significance of faecal eggs/oocysts per gram (EPG/ OPG) means amongst the parasites, villages and between the wet-hot and dry-cold seasons. Lastly, generated PCR data were used to calculate the prevalence based on presence and absence of the detected helminths and reported in percentages.

3. Results and discussion

The gastrointestinal tract (GIT) associated helminth and coccidian parasites were detected from 28% of the households either as single or concurrent infections for the entire period of the study (Fig. 2.). Out of 462-pooled faecal samples analysed, 131 samples showed the presence of chicken gastrointestinal parasite infections. The overall rates of nematode infections were as follows: *A. galli* at 10.4%, *H. gallinarum* at 5%, while a single egg of *C. obsignata* was detected in one flock from Thabang village (Fig. 3). The *E. tenella* recorded 12.8% prevalence with OPG determined to be 1555 and 15,000 in cold-dry and hot-wet seasons, respectively (Fig. 4). A total of 21 adult worms were obtained from five of the six chickens that were incidentally found dead during the sample collection and were identified as *A. galli* (Supplementary Fig. S1).

The above-mentioned GIT parasites were previously reported in free-range chickens in South Africa, which is the only neighbouring country to Lesotho (Thekisoe et al., 2003; Malatji et al., 2016a). The overall prevalence of adult *A. galli* (10.4%) obtained in this study is lower when compared to previously published prevalences of 37.5%, 33.3% and 18.7% in Nigeria, India and South Africa respectively (Ogbaje et al., 2012; Tanveer et al., 2015; Malatji et al., 2016a). The lower prevalence could be due to unfavourable environmental conditions in Lesotho. In winter, the mean temperature is 7 °C with average maximum and minimum of about 15 °C and 0 °C respectively. The higher altitudes are coldest with a mere increase of 300 m in height leading to a decrease of

Table 1
Evolutionary divergence of *Ascaridia galli* representing 367 bp and expressed as standard error (bottom left) and pair-wise distance (p-distance) (top right).

Accession number and geographic location	1	2	3	4	5	6	7	8	9
MN207069.1 Lesotho		0.02	0.02	0.02	0.03	0.03	0.02	0.02	0.01
MN207070.1 Lesotho	0.01		0.00	0.02	0.03	0.03	0.02	0.02	0.02
KT388440.1 South Africa	0.01	0.00		0.02	0.03	0.03	0.02	0.02	0.02
KT388438.1 South Africa	0.01	0.01	0.01		0.04	0.03	0.02	0.01	0.02
MW243594.1 Ghana	0.01	0.01	0.01	0.01		0.00	0.03	0.03	0.03
KP982856.1 Italy	0.01	0.01	0.01	0.01	0.00		0.03	0.02	0.02
GU138670.1 Denmark	0.01	0.01	0.01	0.01	0.01	0.01		0.01	0.02
LC592840.1 Bangladesh	0.01	0.01	0.01	0.01	0.01	0.01	0.01		0.02
OL457052.1 South Africa	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	

temperature by 1.7 °C (Grundling et al., 2015). Moreover, severe frost and snowfalls take place from May to September due to these extreme temperatures (Grab and Nash, 2010). January is the hottest month (37 °C) with the mean temperatures ranging around 21 °C, with average maximum and minimum of about 25 °C and 15 °C respectively. These varyinng conditions affect the viability, distribution, behavior of parasites and lead to reduced chicken encounter with parasites (Kołodziej-Sobocińska, 2019).

The variance can also be attributed to host resistance against gastrointestinal parasites, management system, and year of the study (Gauly et al., 2008; Butboonchoo and Wongsawad, 2015).

The prevalence of *H. gallinarum* detected in the current study (5%) was lower than the previously recorded infection range between 8 and 94% in South Africa; 40% in Nigeria and 56% in Egypt (Mukaratirwa and Khumalo, 2010; Ogbaje et al., 2012; Aziz, 2016). On the contrary, these prevalences are higher than findings by Kumar et al. (2015) in India where they recorded a prevalence of 3.45%. The overall prevalence of *E. tenella* (6.5%) is lower as compared to the previously reported prevalence of 18.6% and 13–15% in Nigeria and South Africa respectively (Jatau et al., 2012; Malatji et al., 2016a).

There was significant difference in prevalences of the three parasites, at $p < 0.05$. The prevalence for *A. galli* and *H. gallinarum* were comparatively higher during the hot-wet season (7.1% and 2.8%, respectively) than the cold-dry season (3.2% and 1.9%, respectively) and varied significantly ($p < 0.05$). For *E. tenella*, the prevalence was slightly higher in the cold-dry season (6.7%) than the hot-wet season (6.2%) (Fig. 3). The parasite counts were highest in wet-hot season (Fig, 4) with *E. tenella* recording OPG value of 8950, followed by *A. galli* and *H. gallinarum* with EPGs of 6950 and 2700, respectively. The spike in egg and oocyst counts could be attributed to increased moisture and warmth due to rainfalls that take place during January to February months in Lesotho. The moisture favours development and accumulation of infective larvae watering and feeding areas (Wuthijaree et al., 2019). On the contrary, prevalence of *A. galli, H. gallinarun* and *E. tenella* did not differ significantly at ($p > 0.05$) amongst the four villages for the entire duration for the study.

Although PCR detected the presence of *Heterakis* spp. and *Capillaria* spp. from pooled DNA samples and different growth-stages of worms that were collected from the intestinal tract, the resultant sequences were of poor quality and therefore could not be compared with published *Heterakis* spp. and *Capillaria* spp. sequences. The adult worms were identified as *A. galli* by microscopic and PCR techniques. The PCR amplified DNA from six (29%) adult *A. galli* worms of which two amplicons only were successfully sequenced and deposited in NCBI GenBank. One of the Lesotho sequences, (MN207070), displayed 100% identity with OL457051, KT388440 and OL457058, while another one (MN207069) showed 100% identity with KT388439 sequences from South Africa in the phylogenetic tree (Fig. 5) based on COX1 partial gene sequences.

The *A. galli* isolates from this study clustered in two separate well-supported clades with South African *A. galli* isolates. According to the evolutionary divergence estimates, our results showed a slight genetic divergence ($p = 0.02$) between the two isolates (Table 1), with both isolates being identical to *A. galli* haplotype VI and VII from South Africa ($p = 0.00$) according to Malatji et al. (2016b), respectively. This clustering and genetic diversity may indicate that there is a possibility of having diverse *A. galli* populations in Lesotho. An infection with *A. galli* can cause deleterious effects on production and productivity of village chickens ranging from decrease in host weight, haemorrhagic enteritis, anaemia, diarrhoea, to death in severe or concurrent infections (Permin and Hansen, 1998). Lack of proper housing, inaccessibility of veterinary services and malnutrition were some of the observed challenges at the selected study areas. The similarity between the Lesotho and South African isolates suggests that similar control strategies may be used by both countries. The finding is particularly important since South Africa is the supplier of poultry and its products to Lesotho.

4. Conclusion

This study has successfully documented the occurrence of *A. galli*, *H. gallinarum*, *E. tenella* and *C. obsignata* in free-range village chickens in four districts of Lesotho using microscopic methods. The *E. tenella* prevalence was highest, followed *A. galli*, and *H. gallinarum*. Generally, the OPG/EPG counts of *E. tenella*, *A. galli* and *H. gallinarum* were highest in March while *C. obsignata* was only detected at Thabang village in September. The high spike of the parasite EPG during March through to April could be attributed to rainfalls that take place during January to February months in Lesotho (Central Bank of Lesotho, 2017). This is the first scientific study to characterize nematodes and coccidian species infecting free-range chickens in Lesotho. Data obtained from this study will contribute to formulation of parasite control strategies for free-range chickens in Lesotho.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2022.100798.

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Ethical consideration

The research proposal was submitted to and approved by Tshwane University of Technology Faculty Committee for Research Ethics-Science and Animal Research Ethics Committee before the commencement of the study.

CRediT authorship contribution statement

Mabusetsa J.R. Makalo: Investigation, Writing – original draft, Funding acquisition, Data curation. **Khethiwe Mtshali:** Conceptualization, Supervision, Writing – review & editing. **Ana M. Tsotetsi-Khambule:** Conceptualization, Supervision, Writing – review & editing. **Lehlohonolo S. Mofokeng:** Software, Formal analysis. **Moeti O. Taioe:** Software, Formal analysis. **Oriel M.M. Thekisoe:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors have no conflicts of interest that might unduly influence their work.

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