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Current status and related risk factors of abomasal nematodes in calves using Mini-FlOTAC as a new technique in Mosul city, Iraq

Hassan Hekmat Shihab
Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq
Corresponding author email: hassan.20vmp44@student.uomosul.edu.iq

Sadam Dhahir Hassan
Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

***Abstract*---**With ongoing climatic alternation, ignoring the influence of repellent resistance, and lack of planned treatment in many countries, the intensity and impact of gastrointestinal nematodes GIN were dramatically increased. The Mini-FLOTAC method for laboratory analysis is presented in the current investigation and is initially suggested to estimate the prevalence of abomasal nematodes and related predisposing factors in calves in Mosul, Iraq. Between October 2021 to the end of April 2022, a cross-sectional study involved several areas, and 480 fecal samples were examined. Animal data, and observed clinical signs documented during sampling. The outcome of this work indicates that the total prevalence of GIN was 50.6%. The infection rate of abomasal nematodes Haemonchus spp., Ostertagia spp., and Trichostrongylus spp. was 62.13%, 60.9%, and 54.73%, respectively. The findings related to epidemiological risk factors demonstrated that animals aged <1 year and herd size >40 were more likely to be infected with abomasal nematodes, whilst gender, origin, and zone characteristics had no significant influence. Ultimately, calves in Mosul are frequently infected with abomasal nematodes of veterinary interest (Haemonchus and Ostertagia spp.). These nematode distributions are clearly influenced by the animals' ages and the size of their herds. For a control effort, it's critical to consider the efficacy of scheduled treatment and be aware of the types of parasite, epidemiology, and presence of ant-helminthic resistance.

***Keywords*---**Prevalence, Haemonchus, Ostertagia, Riskfactors, Mosul.

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Introduction

Numerous nematode parasites can infect the gastrointestinal tract (GIT) of cattle and induce clinical and sub-clinical diseases which influence the health status and cause remarkable financial losses to the farm industry (Njonge,2017). For authorized successful control programs, scanning the animals for helminths infestations is necessary to be performed (Income *et al.*,2021). The majority of gastrointestinal parasites belong to the superfamily Trichostrongyloidea, order Strongylida, phylum Nematoda and include about twenty species. Generally, the species are site-specific and are located in either abomasum, small intestine, or large intestine. It is known that most species are host specific and parasitize on livestock alone although transmission can occur between sheep and other ruminants. There are many genera number of species in the superfamily Trichostrongyloidea such as Trichostrongylus, Haemonchus, Ostertagia, Nematodirus, Marshallagia and Cooperia and they are parasites primarily endemic to the digestive system of animals and spread throughout the world. Trichostrongyloid nematodes (Jackson, 2013; Hosseinnezhad *et al.*, 2021).

Most types of gastrointestinal worms have a simple and direct life cycle. Usually, the eggs hatch in the feces of infected animals into first-stage larvae (L1) in the dung, then complete two more molts to L2 and then L3, which represents the infective stage (Hoste and Torres-Acosta, 2011). The infective L3 migrates away from the feces to nearby plants where it is inadvertently ingested by grazing livestock. Once in the host, the larvae undergo a further molting process into fourth and fifth stage larvae (L4, L5) which finally develop into adult worms to complete the life cycle, which takes about 21 days in the host. Many types of roundworms go into hibernation, a process known as hypobiosis, during the winter months, which time cattle will not shed their eggs (Gibbs, 1982; Gatongi *et al.*, 1998).

At the regional level, climatic and environmental factors and management conditions vary and may lead to important differences in the level of exposure to gastrointestinal nematodes between countries (Bennema *et al.* 2010). Gastrointestinal roundworm infection is a limitation of efficient livestock farming worldwide. Especially in less developed agricultural systems, parasitic infections may cause severe clinical signs, such as stunted growth, tissue edema, and diarrhea. Even in well-managed herds with no signs of clinical parasitism, the presence of gastrointestinal parasites stunts growth in young animals and reduces milk production in adult cows (Gross *et al.*, 1999; Gasbarre *et al.*, 2001).

The epidemiology of GIN infection in cattle may vary in a given geographical area depending on climatic conditions and management systems (e.g., grazing management and deworming use) (Charlier *et al.*, 2020). In Canada, for example, some changes have occurred over the past two to three decades in the prevalence of gastrointestinal helminth infections and beef production due to higher annual and seasonal temperatures, fluctuations in herd size, and lower antihelminthic efficacy (Avramenko *et al.*, 2017). Several studies have reported that parasite infection of the digestive system of ruminants is caused mostly by roundworms and trematodes. However, the types of diseases and the prevalence of parasites among animal groups are greatly influenced by geographic location and seasons.

Furthermore, climatic conditions such as ambient temperature and precipitation patterns have a significant impact on the rangelands and the cycle of food resource availability throughout the year (Ntonifor *et al.*, 2013).

The principal effort to the control of gastrointestinal nematode infection is treatment of the entire herd. The anthelmintic treatment can be repeated at frequent intervals, or broad-spectrum repellents can be used (Maqbool *et al.*, 2018). Comprehension the epidemiology of gastro-intestinal nematodes such as FEC density, mode of transmission, and predisposed factors may not considered in routine treatment strategies for whole herd, and thus, treatment may not occur at times when treatment efficacy must be maximized. Additionally, cattle are not qualitatively or quantitatively screened for GIN infection to make a decision on whether or not to treat (Wills *et al.*, 2020). Thus, this approach is likely to be less efficient compared to targeted selective treatment. Currently, routine blind treatment is not recommended for livestock grazing operations, as this method of treatment may increase some drawbacks such as drug resistance of the worms (Sutherland and Leathwick, 2011).

Fecal egg count FEC methods for gastro-intestinal nematodes are the most widely used diagnostic method in cattle. Fecal egg counting methods require minimal technical expertise, are cost-effective, and can be used in field assessment for GIN infection. Estimation of infestation severity, evaluation of anthelmintic efficacy, and use as a guide for GIN treatment and control programs are some of the reasons for applying FEC as a diagnostic method (Eysker and Ploeger, 2000; Roeber *et al.*, 2013). Mini-FLOTAC is a new method for determining FEC and it is a modified version of the FLOTAC method, which includes direct centrifugal flotation technology. The minimum detection limit for mini-FLOTAC is 5 EPG. However, Mini-FLOTAC is based on gravity flotation egg flotation technology and has greater potential to detect GIN eggs than McMaster and modified Wisconsin flotation (Barda *et al.*, 2013; Paras *et al.*, 2018). This method is relatively fast and suitable for evaluations in the field and in laboratories with a minimum supply of equipment since the presence of a centrifuge is not a mandatory requirement. Mini-FLOTAC has high accuracy and sensitivity compared to modified Wisconsin and McMaster methods; Therefore, it was recently proposed as an appropriate test in the proposed guidelines for FECRT in cattle (Kaplan, 2020).

As there is scanty epidemiological information on the prevalence of abomasal nematodes in Mosul city, Iraq, the objectives of this study were: to determine the prevalence of abomasal nematodes in calves. recording the most important clinical signs in infected animals and verifying the relationship of some epidemiological factors to the prevalence rate.

Materials and Methods

Animals and study location

Through a cross-sectional survey of several zones in Mosul from October 2021 to the end of April 2022, 480 calves representing 35 herds were examined. The animals' ages were (<1 year and >1 year), local and imported origins, small herds <40 and large herds >40 calves, and their gender, males and females, were all noted. The managers provided the epidemiological information and the case history, and during the sampling process, the most significant clinical indications were noted in a Clinical Card.

Samples

Fecal samples (480 samples) were collected from the rectum using sterile rubber gloves. The samples are marked and 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.

Laboratory analysis
Fecal egg counts

Fecal egg counts were done by employing a Mini-FLOTAC technique for the first time in this study zone and briefly: Two grams of fecal samples add to 38 ml of saturated salt solution. The screw-top was moved to make the slurry completely homogenized, and then the two counting chambers in the disc were filled after filtering the slurry by the filter on the top cover then left the disc for ten minutes horizontally and then transferred it to the microscope to be examined with a power of 100x magnification (Cringoli *et al.*,2017; Amadesi *et al.*,2020). Eggs per gram were calculated using the following equation:
EPG = (total eggs of two chambers) x 10

Statistical analysis

The prevalence of gastro- intestinal nematodes in calves was calculated using descriptive statistics using Excel 2010 for Windows 10, and the odds ratio was analyzed for epidemiological risk factors (age, herd size, origin, breeding areas and animal sex) using the chi square test In the SPSS program for Windows (version 21, IBM SPSS, USA), All results were considered significant at the $P<0.05$ level

Results

The results of the current study showed through examining 480 fecal samples collected of calves of both sexes and of different ages, and from different origins in a cross-sectional survey of different areas from the right and left side of the city of Mosul, that the total prevalence of gastrointestinal nematodes in calves was 50.6% depending on the FEC and the number of eggs per gram feces EPG were obtained using the Mini-FLOTAC method. (Table 1).

Table 1: Total prevalence of GIT nematodes in calves by Mini-FLOTAC

Method	Total No. tested	No. of +ve Animals (%)	No. of -ve Animals (%)
Mini-FLOTAC	480	243 50.6%	237 49.4%

The results of the study also showed that the majority of fecal samples examined for calves were mixed infection with gastrointestinal nematodes 78.6%, while the rate of single infection was 21.39%. (Table 2).

Table 2: Types and percentage of infection of GIT nematodes in calves

Type of infection	Number	Percentage (%)
single	52	21.39 %
mixed	191	78.6 %

The infection rate of abomasal nematodes Haemonchus spp., Ostertagia spp. and Trichostrongylus spp. was 62.13%, 60.9% and 54.73%, respectively. The infection rates for other intestinal nematodes species Oesophagostomum, Cooperia, Chabertai spp, Bunostomum, Stongyloides and Nematodirus were 19.34%, 32.09%, 9.05%, 8.64%, 0%, 15.22%, respectively. (Table 3). Current study also revealed highest prevalence rate recorded in April, March and February 63.33%, 61.19% and 59.64%, respectively, and the lowest prevalence was recorded in January, 33.33%. (Figure 1).

Table 3: Infection rate of different GIT nematodes species in calves

Nematode species	No. of sample	No. +ve sample	Percentage
Haemonchus	480	151	62.13 %
Ostertagia		148	60.9 %
Trichostrongylus		133	54.73 %
Oesophagostomum		47	19.34 %
Cooperia		78	32.09 %
Chabertai		22	9.05 %
Bunostomum		21	8.64 %
Stongyloides		0	0 %
Nematodirus		37	15.22 %

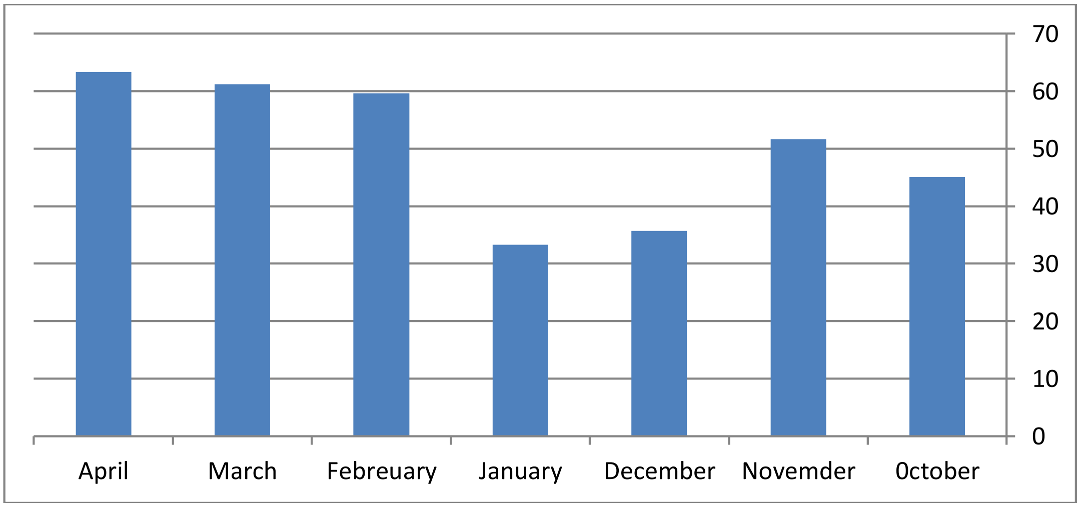

Fig. 1: Prevalence of GIT nematodes in calves according to the months

The affected animals with gastrointestinal nematodes showed clinical signs represented by loss of appetite, pallor mucous membranes, weakness, cough, nasal mucous secretions, diarrhea, loss of hair luster and bottle jaw. (Table 4).

Table 4: Frequency and percentage of clinical sign in infected calves with GIT nematodes (n= 243)

Clinical sign	Frequency	Percentage
loss of appetite	*98*	40.32
pallor mucous membranes	*94*	38.68
weakness	*32*	13.16
cough	*29*	11.93
nasal mucous secretions	18	7.4
diarrhea	*58*	23.86
loss of hair luster	*31*	12.75
bottle jaw	*12*	4.93

The findings related to epidemiological risk factors showed that the age-related factor had a significant effect (P<0.05) on the prevalence of gastrointestinal worms in calves. Animals with an age of <1 year were more at risk to infected with GIT parasites compared to animals >1 year, (odds Ratio = 2.56, Cl: 1.5222-4.3218), P= 0.038. (Table 5). The results also indicate no significant difference (P<0.05) in the prevalence of gastrointestinal parasites relying on the sex of the animal, the infection rates in males and females counted 52.45% and 40.27%, respectively, with a probability of risk (odds Ratio = 1.6356, Cl: 0.9827 - 2.7223), P= 0.3. (Table 5).Furthermore, the results of this work manifested no significant difference (P<0.05) in the prevalence of gastro-intestinal worms based on the origin of the animal, infection rates in imported and local calves were 51.5% and 48.73%, respectively, (odds Ratio = 1.1194, Cl: 0.7648 -1.6383), P= 0.80. (Table 5).

The outcome of our study demonstrated a significant difference (P<0.05) in the prevalence of gastrointestinal parasites counting on the herd size. Respectively, animals >40 heads were more likely to be infected than herds <40 heads, (odds Ratio = 1.8819, Cl: 1.2283 - 2.8833), P= 0.043. (Table 5). Finally, the consequence of the present study indicate no significant difference (P<0.05) in the prevalence of gastrointestinal parasites in calves between the right and left side zone of Mosul city, where the infection rates were 49.53% and 51.5%, respectively, (odds Ratio = 1.0821, Cl: 0.7549-1.5510), P= 0.8. (Table 5).

Table 2: Odds ratio of associated risk factors with the prevalence of GIT nematodes in calves

Factors	No. case tested	No. of +ve (%)	Odds ratio	CI	P
Age					
>1year	76	24 (31.57%) [a]	1		
<1 year	404	219 (54.2%) [b]	2.56	1.5222-4.3218	0.03

Gender					
Male	408	214 (52.45%) [a]	1		
Female	72	29 (40.27%) [a]	1.6356	0.9827-2.7223	0.313
Origin					
Imported	322	166 (51.5%) [a]	1		
Local	158	77 (48.73) [a]	1.1194	0.7648-1.6383	0.803
Herd size					
<40	*116*	42 (36.2%) [a]	1		
>40	*364*	201 (55.21%) [b]	1.8819	1.2283-2.8833	0.043
Area					
Right zone	214	106 (49.53%) [a]	1		
Left zone	266	137 (51.5%) [a]	1.0821	0.7549-1.5510	0.868

Different letters (a, b or c). mean significant difference,($P< 0.05$), CI: Confidence interval, P:probability.

Discussion

For both small and big farms, parasitic gastrointestinal infection is a problem on a worldwide scale. Reduced feed intake, poor growth and weight gain rates, exclusion and slaughter, reduced reproductive efficiency, reduced production capacity, treatment and management costs, as well as mortality of infected animals, are just a few of the ways that parasite infections of ruminants' digestive systems can result in severe economic losses. (El- Ashram *et al.*, 2017).

Findings of this work revealed the remarkable prevalence of gastrointestinal nematodes in calves in Mosul, Iraq, and was 50.6%, by fecal examination using Mini-FLOTAC. Furthermore, 78.6% of the specimens were a mixed infection. This upshot might match or deviate from earlier studies in Iraq and other countries worldwide. In Mosul, (Abdulhameed *et al.*, 2012) indicated that the total percentage of infection with GIT parasites in beef calves was 60.99%, represented by Ostertagia spp, with the highest rate of 61.62%, Haemonchus spp and Trichostrongylus spp 40.69%. and 15.11%, respectively. Another study (Khalaf and Hasan, 2019) stated that the total prevalence of GIT nematodes in cattle was 23.34%, with the highest percentage being Haemonchus spp. 27.13%, followed by Ostertagia spp 26.39%. in Mosul. And in Sulaymaniyah Governorate, Iraq, (Aram,2020) mentioned in his study that the prevalence of GIT in cattle was 18.60%. In Egypt, (Al-Aboody and Omar, 2016) recorded a 30% total prevalence in cattle, and the infection rate was in Haemonchus spp. and Ostertagia spp. 26.5 and 40.9%, respectively. In Somalia (Ghanem *et al.*, 2009) indicated that the total infection rate of gastro-intestinal nematodes was 30.9%, with the highest parasitization rate for Trichostrongylus spp. 22.9% and Haemonchus spp. 2.1%. In Iran, (Kordi *et al.*, 2019) reported 81.25% total prevalence of abomasal nematodes with infection rate of Haemonchus spp. 13.25% and no Ostertagia spp was recorded in cattle. In Nigeria, total prevalence in cattle was 21.9%, of which nematodes were 14%, of which Haemonchus spp and Trichostrongylus spp represented 3.3 and 4%, respectively (Abraham *et al.*, 2020). Various reason could attributed to the difference in rates of infection less and/or higher or near to this study and the studies conducted locally and other countries such as: geographical area, season, numbers of animals examined, techniques used for diagnosis, breeding systems, animal breed And the presence of resistance to repellents. The outcome here agreed with what was recorded by a number of literatures in different countries around the world (Rupa and Portugaliza,2016; Income *et al.*, 2021).

In this study the highest prevalence rate was recorded in the months of April, March and February 63.33%, 61. 19 % and 59.64%, respectively, and the lowest prevalence was recorded in January 33.33%. This disparity among months and seasons may account for the optimum temperature and moisture that supports the growth and development of larvae on pasture and allows animals to graze. This vision is in agreement with the findings of researchers (Gillandt *et al.*, 2018;Balicka-Ramisz *et al.*, 2019; Ola-Fadunsin *et al.*, 2020). Current study also demonstrate that the infected animals display varying severity of clinical signs, represented by partial loss of appetite, pallor of the mucous membranes, general weakness, coughing, presence of nasal mucous secretions, diarrhea, loss of hair of its natural luster and bottle jaw. These manifestation correspond with the conclusions of (Abdulhameed *et al.*, 2012; Aliyara *et al.*, 2012; Karshima *et al.*, 2018). It is possible that these signs are attributed to the poor functioning of the gastrointestinal tract and its lack of movement, as well as the effectiveness of blood absorption by parasites from the host's intestines, which may lead to anemia and protein deficiency. Damage caused by parasites to the intestinal wall of inflammation and necrosis may result in varying degrees Severe diarrhea as well as loss of appetite that may lead to easy pulling and loss of luster. It has been indicated that depending on the number and species of worms, clinical signs of GIN parasitism include loss of appetite and diarrhea. anemia, edema, reduced fertility, and increased exposure to other pathogens (Cheru *et al.*, 2014; Ola-Fadunsin, 2017).

In present study the animals with an age <1year were more likely to get infected with GIT nematodes comparing with >1year animals odds Ratio = 2.56. This conclusion be through with earlier research publications (Kabaka *et al.*, 2013; Gillandt *et al.*, 2018; Haile *et al.*, 2021). The reason here may be rely on the fact that infection with GIT worms, especially in adult animals, tends to be subclinical,. In addition, slow immune response at the time of infection in young animals so are more susceptible, or not yet been treated with different types of repellents compared to older animals, furthermore may be due to limited prior exposure and immaturity of the immune system resulting in a higher development of the parasite. This finding is in line with our results. However, the reasons for the differences prevalence in different age groups could not easily elucidate, but it may related the immune status of the animals, disparity in grazing area, and management (Haile *et al.*, 2021). In contrast, our result did not fall in with (Muktar *et al.*, 2015), who indicated that there was no significant difference between the different age groups in their study.

The findings show that there is no difference in prevalence rates between the sexes of calves. This outcome is in line with that demonstrated by (Shenba *et al*., 2016; Khalaf and Hasan, 2019). The availability of the same exposure chances, similar grazing and management practices, and exposure to the same climate are only a few of the many variables that influence the degree and scope of parasitism. Our findings did not agree with some earlier research. (Habtemichael *et al*., 2018) found a statistically significant difference in infection rates between the sexes, with males having a greater prevalence rate than females, which they attribute to males being more exposed to grazing regions.

The study's findings also demonstrated that there is no significant difference in the prevalence of GIT worms in local and imported calves. These outcomes matched (Ola-Fadunsin *et al*., 2020). In addition to the fact that the imported animals are from countries where the spread of these worms is proven and documented, it is also known that exposure to the digestive worms is common worldwide and exists as long as there is animal husbandry or even in any place where there are animal gatherings. These notes is in line with what other references suggested (Nazish *et al*., 2021). In contrast to this finding, (Bacha and Haftu, 2014), found that imported animals had a higher rate of parasitic worms than local animals and this difference was explained by the high resistance of local cattle breeds and crossbred cattle from pure exotic breeds.

The current study's findings revealed that the larger herds were more susceptible to infection with GIT worms, Odds Ratio = 1.8819. The study's findings corroborated the presumptions (Pinilla León *et al*. 2019). It can be explained by the possibility that immunosuppression in animals can result from crowd-related stress and herd migrations. Additionally, dense herds can raise the level of pasture pollution, which results in increased rates of dissemination (Habtemichael *et al*., 2018). The larger and more numerous the animals, the greater the likelihood that they will be exposed to parasites and eggs on contaminated pastures. The high prevalence of GIN and its spread in the livestock can be related to environmental factors such as temperature and humidity, moreover, breeding density, ineffective management, and inadequate rearing systems are another factors that facilitates the transmission of the parasite (Zvinorova *et al*., 2018;Zulfikar *et al*., 2018).

The study's findings confirmed no significant difference in the infection rate between the right and left sides of Mosul. This finding may be explained by the similarity of the climates, grazing systems, management practices, and strategies employed in the management of digestive worms. According to Kabaka *et al*. (2013), the explanation for the discrepancy is that some farmers strengthened management efforts, such as breeding place, feeding, and helminth control. These data, however, do not corroborate their findings. A decrease in GIN infection could have been the result of this management improvement. The current findings also conflicted with those of (Haile *et al*., 2021) who explain the reasons to the variations in animal populations, repellent usage tactics, and management approaches between the study regions, as well as the presence of varied ages and mixed grazing.

Conclusions

The results of this research suggest that abomasal nematodes are common in calves in Mosul, in the north of Iraq, and can be seen at varying rates in other countries across the world. Age and herd size are the main confounders associated factors. Additional epidemiological research is required to assess the severity of the illness and its financial implications.

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Conflict of interest

No conflicts of interest are disclosed by the authors of the work.

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