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Detection of resistance against anti-helminths drugs in gastrointestinal nematodes of calves using fecal egg count reduction test FECRT

H.H. Shihab and S.D. Hassan

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

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Correspondence:
S.D. Hassan
hasanali@uomosul.edu.iq

Abstract

The species of parasite, low dose, and continual employ of the same drug may predispose to the evolution of anthelmintic resistance AR. In Mosul, Iraq, this is the first study investigating AR in gastrointestinal GIT nematodes of calves. Four hundred eighty fecal samples through a cross-sectional survey were examined using the Mini-FLOTAC. A herd of calves consisting of 60 male calves was divided into four groups of 15 calves: group A counted as control, group B was treated with Reemazol® 25% (Albendazole) 7.5 mg/kg of body weight orally, group C received Dufazan® (Levamisole and Oxyclozanide) 7.5 mg/kg BW orally, and group D gave Ivermectin 1% by S/C injection 0.2 mg/kg BW. The efficacy asset employs the Fecal Egg Reduction Test (FECRT). AR judgment obtains relying on the instructions of the World Association of advancement for Veterinary Parasitology (WAAVP). GIT nematode prevalence was 50.6%. The effectiveness of mentioned drugs was 84, 87, and 95%, respectively. The lower limit confidence interval 95% level was 89, 86, and 80%, respectively, indicating AR to albendazole and levamisole, while ivermectin was questionable. In conclusion, the high prevalence of GIT nematodes in Mosul indicate that AR is present against the three classes of deworming drugs. Awareness of the associated aspects and sources of resistance is essential to face and minimize the development of resistant worms.

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Introduction

Gastrointestinal parasites negatively influence livestock productivity and have an essential role in the economic losses incurred by animals due to the cost disease treatment, mortality, reduced fertility, growth rate, weight loss, poor nutritional metabolism, loss of appetite, anemia, and diarrhea (1-3). The *Haemonchus spp, Trichostrongylus spp,* and *Cooperia spp*, belong to the family Trichostrongylidae; *Oesophagostomum spp* belongs to the family Strongylidae and *Strongyloides spp* of the family Strongyloididae, and *Trichuris spp*. of the family *Trichuridae* are the main genera of nematodes that parasitize the gastrointestinal tract of cattle. Parasitic infection usually occurs with optimal temperatures and humidity, and transmission occurs during ingestion contaminated pastures with the eggs. Infective larvae that become inside the digestive system of adults, reach maturity, multiply and release eggs into the environment through feces. Moreover, more than one species of these parasites may infect and parasitizes the same animal (4-6). Various strategies are implemented to control gastrointestinal nematode infestation in animals' population, practically deworming medicines are the standard prevalent mode of helminths control, and treatment of the entire herd through by either repeated anthelmintic at frequent intervals or the use of broad-spectrum anthelmintic drugs is the predominant preventive approach to control nematodes infection globally. Among the most common safety, broad-spectrum anthelmintic used drugs are benzimidazoles, Macrocyclic lactones, and imidazothiazoles (7,8), the consequence of their widespread use enhances the development of anthelmintic resistance (AR), which occurs when the recommended drug dose is not able to It effectively treats the infected animal (7-10). Awareness of gastrointestinal nematode epidemiology, fecal egg count density, mode of transmission, and predisposed animals should consider when adopting systematic treatment strategies for whole herds. Conversely, treatment may not occur at times when it is a priority. Therefore, a blind routine treatment in cattle is not recommended because this method may encourage disadvantages such as drug resistance (11,12). Resistance to one anthelmintic class, or even to several or all classes of anthelmintic, may arise, which can be referred to as multidrug resistance. The anthelmintic resistance seems to correspond to the popularity of anthelmintic classes used in veterinary practice (13,14). Anthelmintic resistance can be defined as a heritable event that enable the dropping of sensibility of the deworming drug in one or more classes of parasite populations previously sensitive to the identical anthelmintic Unluckily the comprehensive utilization of anti-helminthic in veterinary practice has given rise to a severe and dramatic level of resistance noticeable in various helminths of nearly all animal species and the diverse classes of anthelmintic worldwide. Identification of anthelmintic resistance can be assessed in vivo by employing methods such as fecal egg count and approved by the World Association for the Advancement of Veterinary Parasitology (WAAVP). Moreover, in vitro procedures include egg hatch assays, larval motility tests, larval development tests, and PCR (15).

In Mosul, Iraq, no previous study indicated anthelmintic resistance occurrence in calves. In this light, the objective of the current study was to investigate anthelmintic resistance in gastrointestinal nematodes of calves based on the reduction rate of the egg in fecal samples using (FECRT).

Materials and methods

Ethical approve

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

Animals and location of the study

A total of 480 calves, representing 35 herds, were included in this study through a cross-sectional survey of several zones in Mosul between October 2021 and the end of April 2022. The ages of calves were (< 1 year and ≥1 year), local and imported origins, small herds less than or equal to 40 calves and large herds greater than 40 calves, and of both sexes, males and females. 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 in which the information was recorded during the sampling process.

Ethical approval

The ethical approval was issued by the Institutional Animal Care and Use Committee (UM.VET.2021.20) of the Faculty of Veterinary Medicine, the University of Mosul, on the 6^{th} of September 2021.

Collection and handling of fecal samples

Fecal samples (480 samples) were collected directly from the rectum using sterile rubber gloves for each sample. 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 College Veterinary Medicine, University of Mosul. Samples that did not examine on the same day were refrigerated at 4˚C to be examined on the next day.

Fecal egg counts

Fecal egg counts for each sample were done by employing a Mini-FLOTAC technique. This method was used briefly for the first time: Two grams of fecal samples were added to 38 ml of saturated salt solution. The screw-top was moved to make the slurry thoroughly 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 (16,17). Eggs per gram were calculated using the following equation: EPG = (total eggs of two chambers) x 10.

Fecal egg count reduction test

For measuring the efficiency of the most common parasitic repellents used by veterinarians and breeders in Mosul city, and during field intervention on calf farms, a herd of calves was selected, comprising 70 calves with an average number of EPG greater or equal to ≥100. 60 male calves aged 9 months were selected, marked, and further categorized into 4 groups, each of 15 calves. Group A was counted as a control group, and the second group (group B) was treated with Reemazol 25 % drug (Albendazole, 7.5 mg/kg body weight) by oral administration, and the third group (group C) was treated with DUFAZAN (Levamisole and Oxyclozanide), 7.5 milligrams per kilogram of body weight) oral administration, the last group (group D) was treated with Ivermectin SPI 1% at a dose of 0.2 mg/kg of body weight by subcutaneous injection in the neck area. Fecal egg count (FEC) and the EPG for each group were calculated on the first day (Day Zero) before treatment and the fourteenth day (Day14) after medication. The egg reduction ratio (FECRT) in feces was calculated from the following equation (18): $FECRT\% = (T1-T2)/T1 \times 100$ in which T1 represents ±EPG before medication, and T2 is the ± EPG after given the drug.

Statistical analysis

The prevalence of gastrointestinal nematodes in calves Statistically analyzed using Excel sheath software for Windows 7. Significant differences in treated and control groups in a ±EPG and analysis of FECRT, lower limit for confidence intervals of 95%, and the Pearson's correlation coefficient between variables were assessed in SPSS software for Windows (version 19; IBM, USA). The final verdict for Anthelmintic resistance is as follows (19,20): [1] The gastrointestinal parasites are sensitive to the anthelmintic if the FECRT ratio is equal to or higher than 95 percent, and the confidence 95% lower limit is ≥ 90%. [2] Worms resist repellents when the egg reduction ratio is under 95%, and the lower limit confidence 95% level does not match 90%. [3] Suspected anthelmintic resistance was found if one of the two criteria was matched.

Results

The present study indicated that the prevalence of gastrointestinal nematodes in beef calves in Mosul was 243/480 (50.6%) depending on the fecal examinations using Mini-FLOTAC methods (Table 1).

Table 1: The prevalence of gastrointestinal nematodes in beef calves using the Mini-FLOTAC method

Method	Tested	+ve (%)	-ve (%)
Mini-FLOTAC	480	243 50.6)	237 49.4)

This study showed that most fecal samples were mixed infection 78.6% and single infection 21.39% with gastrointestinal nematodes, and the prevalence rate of infection with nematodes of *Haemonchus* spp, Ostertagia spp, and *Trichostrongylus* spp were 62.13, 60.9, and 54.73%, respectively. Moreover, the prevalence rate of infection with nematodes of Oesophagostomum spp., *Cooperia*, spp *Chabertai* spp., *Bunostomum* spp, *Strongyloides* spp, and *Nematodirus* spp. were 19.34, 32.09, 9.05, 8.64, 0, 15.22% respectively (Table 2).

Table 2: Number and percentage of infection with types of gastrointestinal nematodes in beef calves using Mini-FLOTAC

GIT nematodes species	Tested	+ve (%)
Haemonchus spp.	243	151 (62.13)
Ostertagia spp.	243	148 (60.9)
Trichostrongylus spp.	243	133 (54.73)
Oesophagostomum spp.	243	47 (19.37)
Cooperia spp.	243	78 (32.09)
Chabertai spp.	243	22 (9.05)
Bunostomum spp.	243	21 (8.64)
Strongyloides spp	243	0
Nematodirus spp.	243	37 (15.22)

The current study showed that the anthelmintic albendazole, levamisole, and ivermectin significantly affected P<0.05 in reducing the EPG of feces in the treated groups on day 14 of treatment. The mean of EPG was 54 ± 58.162, 34 ± 35.617, and 12 ± 16,987 for the above mention drugs, respectively, compared with the EPG for the same groups on Day 0 (Table 3).

This study revealed that the FERCT calculation for the deworming drugs Albendazole, Levamisole, and Ivermectin was 84, 87, and 95%, respectively, while the percentages of the lower limit confidence level of 95% of these anthelmintic drugs were 89, 86 and 80%, respectively (Table 3). According to the World Association for the Advancement of Veterinary Parasitology (WAAVP), the study results indicate that gastrointestinal nematodes are resistant to albendazole and levamisole, while their resistance to Ivermectin is questionable.

Table 3: Anthelmintic resistance of GIT nematodes to the anthelmintic drug in beef calves

Drug	Animal	± EPG (Day 0)	± EPG (Day 14)	FECRT %	CI 95 %
Control	A	298.667±208.973	316±173.032		
Albendazole	B	330±164.838	54± 58.162*	84%	89%
Levamisole	C	264±167.835	34 ± 35.617*	87%	86%
Ivermectin	D	250±142.277	12 ± 16.987*	95%	80%

±mean and standard error of mean, EPG egg per gram of feces, FECRT fecal egg count reduction test, CI confidence interval, * significant difference at P<0.05.

Discussion

Results indicate altitude prevalence of gastrointestinal nematodes in calves in Mosul, Iraq. The occurrence of gastrointestinal nematodes was 50.6%, depending on the fecal examination by Mini-FLOTAC, and 78.6% of the samples were a mixed infection. This result might harmonize or diverge from previous studies in Mosul, Iraq governorates, and other countries worldwide. (21) revealed a 60.99% prevalence rate, represented by Ostertagia spp, with the highest percentage of 61.62%, Haemonchus spp, and Trichostrongylus spp at 40.69% and 15.11%, respectively. In Sulaymaniyah was 18.60% (22). In Iran, it was 81.25%. In Germany, it was 41.1% (23). The reasons that led to a difference in recording different prevalence less or higher with or close to this study and the studies conducted locally and, in some countries, could be region, climate, sample size, laboratory tools, management systems, the origin of animals, And the presence of resistance to repellents. The result of this study is consistent with what was recorded by researchers in different countries worldwide (24,25). It is also to be noted that climatic alteration could affect the recurrence, density, and zone allocation of parasites, which directly affects the phase of their spread in the surroundings, while secondarily on the larvae that live primarily in intermediate families of invertebrates. Global warming biologically diverse nematodes' distribution range modifies their development cycles (26-30).

Results also showed that the anthelmintic used in this study, albendazole, levamisole, and ivermectin, considerably decreased the FEC of feces in the treated groups on Day 14 of treatment in comparison with FEC on pretreatment time. The efficacy of the drug based on the reduction rates of fecal egg counts for the repellents mentioned above were 84, 87, and 95%, respectively. These results are supported by what was referred to in the earliest literature (21,31,32). Usually, severe infestations with nematodes can cause significant economic losses and even death in severe and neglected cases in calves. Therefore, anthelminthic treatments are often given either as a preventive measure to prevent such losses or as a treatment for nematode infection. Therefore, the availability of effective anthelmintic products is of great importance in livestock systems (17,33).

Our finding also revealed that the lower limit confidence 95% level for Albendazole, Levamisole and Ivermectin was 89%, 86%, and 80%, respectively. Based on the recommendation of WAAVP, the results of the study showed that GIT worms are resistant to Albendazole and Levamisole, while their resistance to Ivermectin is questionable.

Generally speaking, in Iraq, the typical three classes of broad-spectrum anthelmintic drugs available and widely used in veterinary practice to control gastrointestinal nematodes in cattle are Albendazole (benzimidazoles), Levamisole (Imidazothiazoles), and macrocyclic lactones. However, the chemopreventive approach to control GIN is threatened by the emergence of nematode-resistant groups of worms, and the development of resistance to these repellents can be attributed to many reasons such as high frequency of treatment, the low wrong dose of drugs, and poor pasture management by breeders. The visions of this study agreed with what was confirmed by Bloemhoff *et al.* (33). A study conducted in Mosul city to assess the effectiveness of Albendazole for handling roundworms in sheep (34) confirmed the existence of resistance of these worms against Albendazole and hypothesized two main reasons for this high prevalence of resistance in their study. Firstly, the frequent use of the same drug. Secondly, Albendazole was the most commonly used and cheapest anthelmintic among other anthelmintics. Furthermore, Sulaymaniyah province in northern Iraq (34,35) mentioned in their study, and for the first time in the province, that intestinal nematodes showed resistance to the anti-helminthic albendazole, ivermectin, and levamisole in sheep fields.

Finally, AR anthelminthic resistance could be explained as the capability of parasites to remain alive and resist the drugs that ordinarily eliminate parasites of the same species. This resistance is inherited, selected, and resistant generations pass resistance genes on to their offspring, with the constant and notable expansion of helminths resistance AR by parasitic worms of the ruminants over the years. The resistance of worms has also been verified in several previous studies and for all classes of anthelmintic available globally, namely benzimidazoles, imidazothiazoles, and Macrocyclic lactones (36-39).

Conclusions

The study concluded that the GIT nematode parasite is prevalent in calves in Mosul, Iraq, and many countries. It could be observed at various rates. This study documented Anthelmintic resistance AR in bovine gastrointestinal nematodes against Albendazole, Levamisole, and Ivermectin. Serious steps should endeavor to overwhelm and minimize the AR, such as using a combination of different anthelmintic classes, more studies are recommended to assess the AR in other parasite species. Technical criteria should be applied for the control of gastrointestinal parasites.

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Conflict of interest

No conflict of interest in this manuscript

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الكشف في مقاومة طفيليات ديدان المعدة والأمعاء الإسطوانية لطاردات الديدان في العجول باستخدام اختبار اختزال عدد البيوض في البراز

حسن حكمت شهاب و صدام ظاهر حسن

فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

قد تؤهب أنواع الطفيليات والجرعات المنخفضة والاستخدام المستمر لنفس الدواء لتطور مقاومة الديدان. في الموصل، العراق هذه هي الدراسة الأولى التي أجريت للتحقيق في مقاومة مضادات الديدان في طفيليات الديدان المعدية المعوية الإسطوانية في العجول باستخدام اختبار اختزال عدد البيوض في البراز. تم فحص ٤٨٠ عينة براز من خلال مسح مقطعي باستخدام تقنية المني فلوتاك. تم اختيار قطيع مكون من ٦٠ من ذكور عجول التسمين بعمر ٩ أشهر وقسمت إلى أربع مجاميع من ١٥ عجلا: المجموعة ا عدت كمجموعة سيطرة، المجموعة ب عولجت بعقار البندازول بجرعة ٧,٥ ملليغرام / كغم من وزن الجسم عن طريق الفم، المجموعة س عولجت بعقار الليفاميزول بجرعة ٧,٥ ملليغرام / كغم من وزن الجسم عن طريق الفم، والمجموعة د عولجت بالحقن تحت الجلد بعقار الايفرمكتين ١% بجرعة ٠,٢ ملليغرام / كغم من وزن الجسم. تم قياس فعالية الأدوية باستخدام اختبار اختزال عدد البيوض في البراز. تم الحكم على وجود مقاومة الديدان اعتمادا على إرشادات للجمعية العالمية للتقدم في مجال الطفيليات البيطرية. بلغت نسبة انتشار الديدان المعدية المعوية الإسطوانية في العجول ٥٠,٦٪. بلغت فعالية الأدوية المذكورة أعلاه ٨٤ و ٨٧ و ٩٥% على التوالي. الحد الأدنى لمستوى ثقة ٩٥% بلغت ٨٩، و ٨٦، و ٨٠%على التوالي مما يشير إلى مقاومة ضد عقاري ألبندازول الليفاميزول بينما كانت المقاومة للإيفرمكتين مشكوكة. استنتجت هذه الدراسة انتشار واسع لديدان المعدة والأمعاء الإسطوانية بين العجول في مدينة الموصل. وجود مقاومة لديدان المعدة والأمعاء الإسطوانية ضد الأصناف الثلاثة الشائعة من طاردات الديدان. يعد الوعي بالعوامل المؤهلة وآليات مقاومة الديدان أمرا مهما لمواجهة وتقليل تطور الطفيليات المقاومة.