Study of gastrointestinal nematodes resistance to some anthelemitics and evaluation the efficacy of condensed tannin extraction on resistant genera in sheep in Babil

H. M. Al-Ramahi
Coll.of Vet. Med. /Univ. of Babylon

Abstract

The study was carried out in Babel province, where three sheep farms evaluated for albendazole, levamisol and ivermectin resistance in nematodes by in vivo and in vitro techniques as well as to demonstrate the efficacy of commercial tannin-rich solution on the resistant genera.According to low confidence interval limit of 95% fecal egg count reduction FECR ,In farm I, the animal which treated with levamisol and ivermectin considered as suspected resistant, while the animals which treated with albendazole showed resistance to it. In farm II ,the animal which treated with levamisol and albendazole were considered as suspected resistant ,while no FECR% <95 % was recorded in animals which treated with ivermectin ,and in the same manner ,the animals of farm III which treated with levamisol and albendazole showed suspected resistance and resistance respectively, and no resistance to ivermectin was recorded in animals which treated with it. Also, the results revealed that the combination between *in vitro* (EHA) and *in vivo* (FECRT) necessary to confirm the assessment of resistance against benzimedazoles. The most common genus identified from pre treatment feces were *Haemonchus* ,*Trichostrongylus* and *Cooperia* ,while *Haemonchus* was the only identified genus which demonstrated from post treatment fecal culture.The compared of *in vitro* effect of condensed tannin CT and thiabendazol revealed that both thiabendazole and CT induced significant egg hatching inhibition in a dose-dependent manner. The thiabendazole required a maximum of 0.5mg/ml, whereas, the CT required a maximum concentration of 2 mg/ml, to induce 100% egg hatch inhibition. The adult worm motility assay showed that the thiabendazole kill all worms at the concentration of 0.25 mg/ml, while CT was showed insignificant killing even in maximum concentration.

Introduction

The gastrointestinal helminthes are a major contributors to reduce animals productivity (1), and the subclinical infestation may lead to about 40% depression in weight gain (2) .*Haemonchus spp* is a highly pathogenic parasite which facing the sheep farms, which capable to causing acute disease with high mortality rate in all age's groups (3).The extensive use of anthelmintics for control of parasitic infestation in sheep and goat has resulted development of resistance to one or more of widely used anthelmintics in many countries, and the facing of this problem begins with epidemiological studies to obtain reliable information on their occurrence and spread (4). Alternative strategies for parasite control are urgently needed, and one approach may be include plants which contains condensed tannins (CT) into grazing ration (5) .Some studies reveal that CT can increase the proportion of dietary protein that reaching the intestine , and this associated with increased the in young sheep (6,7) ,while other authors study the direct effect of CT on the eggs and larvae of gastrointestinal parasite; (8,9,10).The objective of this study was to demonstrate the prevalence of anthelmintics (levamisole, albendazole and ivermectin) resistance in gastrointestinal nematodes in different parts of Babil province and to investigate the efficacy of commercial herbal CT solution on the eggs , larvae and adults of most resistant genus .

Materials and Methods

Animals:

The study was undertaken in three sheep flocks, include flock I (84 heads), flock II (118 heads) and flock III (109 heads) were chosen from northern, eastern and southern Babil respectively. All the studied flocks were practiced a semi – intensive management and housed overnight on dusty –muddy floor pens , all animals in three flocks does not treated with any anthelmintics for at least 1.5 month prior to present study.

Experimental Design

At first visit, thirty lambs were chosen randomly; individual fecal samples were collected from each animal, and placed in air tight plastic containers and placed in cool box until store in refrigerator. Immediately after sampling the lambs are distributed into three equal groups, with unique dye marking for each. The animals of group 1 and 2 were drenched with levamisol (Valbazen, India) and albendazole (Higro, Italy) in dose rate 10mg /kg and 7.5 mg / kg BW respectively while the animals of group 3 were administrated with ivermectin (Ancare, New Zealand) 0.2 mg /kg sub/cut. In days 10-14, the lambs of each group resampled.

Fecal Egg Count Reduction Test (FECRT)

FECRT were carried out with the samples which collected pre and post the treatment by modified McMaster method as describe by (11), the FECR% was calculated by the formula:

FECR% = 1- (mean FEC post treatment / mean FEC pretreatment) × 100

Resistance to an anthelmintics was considered to be present if FECR% was less than 95% and the lower limit of confidence interval of 95% was less than 90% , while the resistance was suspected when only one criteria was present(11) .

Fecal Sample Culture

The fecal culture of pooled samples before and after treatment was performed according to method mentioned by (12), and the larvae were identified as described by (13).

Preparation of Condensed Tannin CT

The CT solution was purchased from private herbal store, which use in traditional treatment under name "Depaque" or "Jefet", its consist mainly from equal parts of aqueous extract of dried outer wall of pomegranate fruit and Mazu *Quercus infectoria* which called locally "Affas", after evaporation of solution, the dried material stored in freeze conditions, then the PBS was added to make serial dilution which used in egg hatch assay EHA and adult worm motility assay.

Egg Hatch Assay

EHA was performed to confirming of benzimidazole and to compare the efficacy of CT with thiabendazole ,its carried out according to method described by (14),briefly by incubation of counted eggs which extracted from pooled each group's post treatment samples in serial dilution of thiabendazole: 0.0625, 0.125 ,0.25 ,0.5 ,1 ,2 and 4 µg/ml in well plate for 24 hours at $26C^0$, 2ml of distilled water was added to control wells. The EHA % was calculated by following formula:

EHA% = (No. of larvae/no. of eggs +No. of larvae) ×100

To compare EHA% between thiabendazole and CT, the previous method was repeated with the same serial dilution for both preparations.

Adult Worm Motility Assay

Adult *Haemonchus* were collected from abomasums of sheep slaughtered in Babel abattoir, the experiment was conducted according to (15).The motility index was calculated as the ratio between the immobile worms/total No. worms in 3 wells per concentration.

Results

Before treatment ,all animals In farm I,II and III had positive egg of gastrointestinal

nematodes ,counts in means of 312 ,256 and 158 egg/ gram ,with incidence rate 42.9% ,35.3% and 21.8 % respectively ,figure1.

Fecal Egg Count Reduction Test (FECRT)

In the three studied farms, the results of FCRT are presented in table 1 and figure2.In farm I the FECR% <95 % in animal which treated with levamisol, albendazole and ivermectin were 30%, 60% and 10% respectively, and according to low confidence interval limit of 95% FECR, the animal which treated with levamisol and ivermectin considered as suspected resistant, while the animals which treated with albendazole showed resistance to it. In farm II ,the FECR % <95 % in animal which treated with levamisol and albendazole were 30% and 80% respectively, the treatment with albendazole and levamisol which evaluated as suspected resistant ,while no FECR% <95 % was recorded in animals which treated with ivermectin ,and in the same manner ,the animals of farm III which treated with levamisol and albendazole showed suspected resistance and resistance respectively, and resistance to ivermectin was recorded in animals which treated with it.

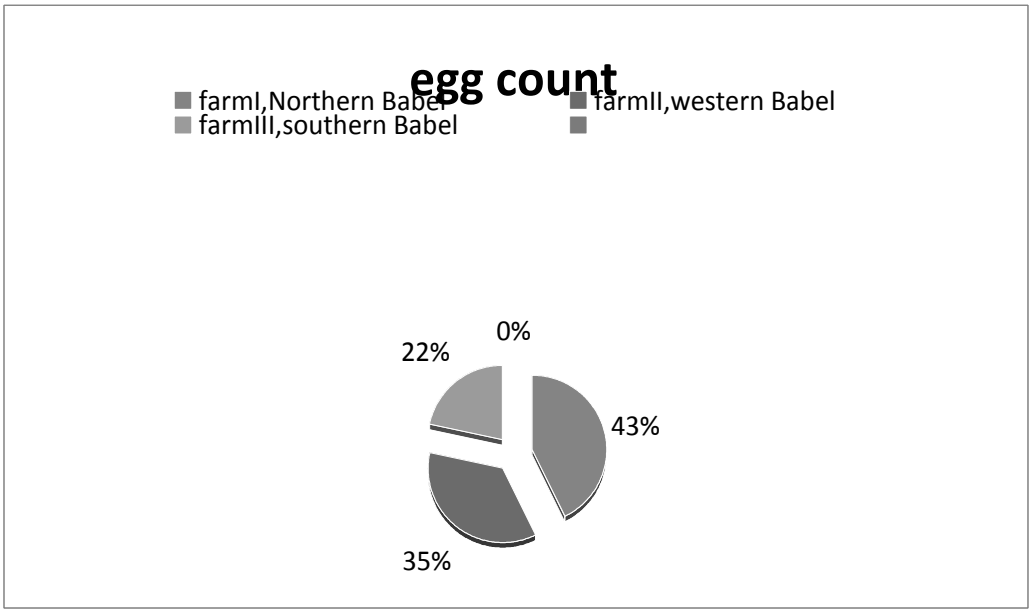

Fig. 1 ,reveal the incidence of gastrointestinal nematodes eggs in studied farms

Table 1 reveal the evaluation of levamisol, albendazole and ivermectin according to FECRT and low CI limit

Farm	anthelmintic	FECR% <95 %	Low CI limit <90 %	Treatment evaluation
I	Levamisol	93.7	93	Suspected
	Albendazole	93.4	87.9	Resistant
	ivermectin	94.4	94.4	Suspected
II	Levamisol	94.1	93.4	Suspected
	Albendazole	92.3	88	Resistant
	ivermectin	-	-	Sensitive
III	Levamisol	93.5	91.3	Suspected
	Albendazole	91.5	87.3	Resistant
	ivermectin	-	-	Sensitive

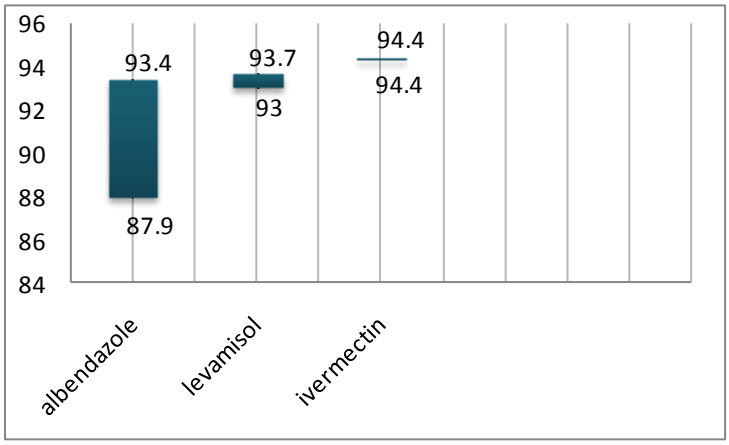

Fig. 2 reveal the FECR %< 95% and low CI limit of farm I

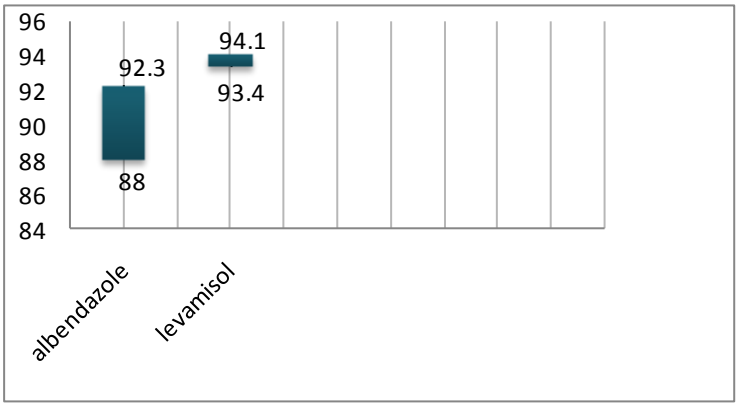

Fig. 3 reveal the FECR %< 95% and low CI limit of farm II

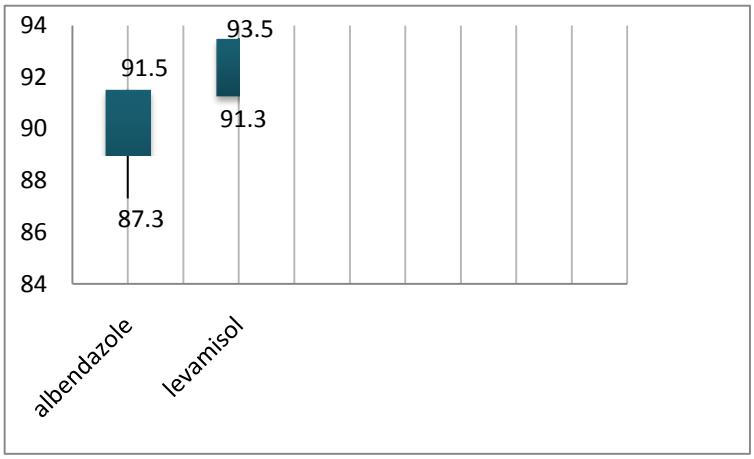

Fig. 4 reveal the FECR %< 95% and low CI limit of farm III

Fecal Culture

The most common genera are identified from pretreatment feces were *Haemonchus* ,*Trichostrongylus* and *Cooperia* in farm I: *Haemonchus* and *Cooperia* in farm II and farm III .After treatment, only *Haemonchus* was present in pooled fecal samples, table 2.

Table 2 reveals the genus of nematodes which recovered from pre and post fecal culture

farms	Before treatment	After treatment
I	*Cooperia,Haemonchus,Trichostrongylus*	*Haemonchus*
II	*Haemonchus ,Cooperia*	*Haemonchus*
III	*Haemonchus ,Cooperia*	*Haemonchus*

Egg Hatch Assay for Thiabendazole

Thiabendazole induced significant egg hatching inhibition in a dose-dependent manner. The maximum concentration of 0.5 mg/ml, required to induce 100% egg hatch inhibition, table 3. Table 4, compares the results of FECRT and EHA with albendazole and thiabendazole respectively, and reveals that the results of both techniques are necessary to obtain complete information about resistance to benzimidazole.

Table 3 reveals the EHA % of thiobendazole

Conc.(µg/ml)	No. of larvae	No. of larvae +No. of eggs	EHA%
0.0652	76	100	76
0.125	62	100	62
0.25	50	100	50
0.5	0	100	0
1	0	100	0
2	0	100	0

Table 4 reveals the results of albendazole FECRT and thiabendazole EHA

farms	FECRT Albendazole	EHA thiabendazole	benzimidazole resistance
I	resistance	resistance	Yes
II	Resistance	resistance	Yes
III	resistance	resistance	Yes

Egg Hatch Assay for Thiabendazole and CT

Both thiabendazole and CT induced significant egg hatching inhibition in a dose-dependent manner. The thiabendazole required a maximum of 0.5mg/ml, whereas, the CT required a maximum concentration of 2 mg/ml, to induce 100% egg hatch inhibition, table 5, figure5.

Adult Motility Assay

The thiabendazole kill all worms at the concentration of 0.25 mg/ml, while CT was showed insignificant killing even in maximum concentration.

Table 5 reveals the EHA of thiabendazole and CT

Conc.(µg/ml)	EHA% of Thiabendazole	EHA% of CT
0.0652	72	98
0.125	58	97
0.25	46	64
0.5	0	55
1	0	13
2	0	0
4	0	0

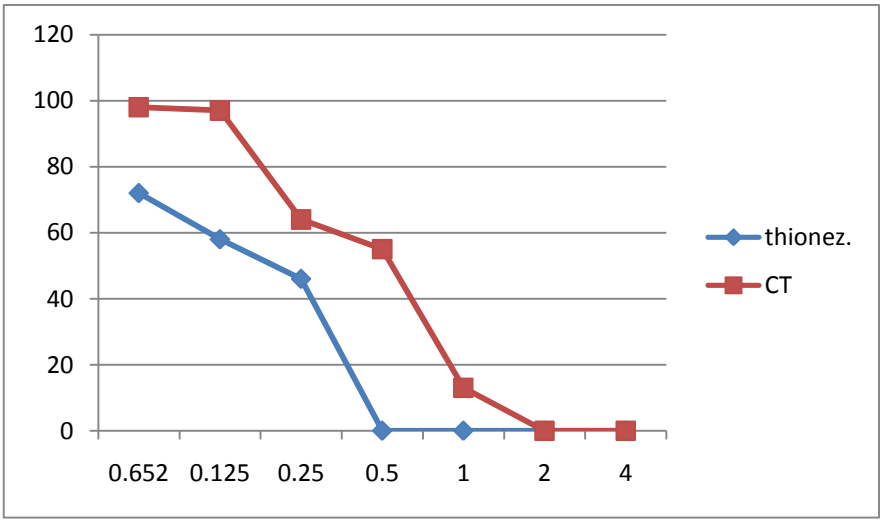

Fig. 5 reveal the EHA of thiabendazole and CT

Discussion

The resistance to anthelmintics seems to have occurred following prolonged and intensive use of it in sheep farms. Albendazole and levamisol are widely used in Iraq since development of veterinary animal care, and different manufacturers sell it under many trade names, and both of above anthelmintics are used traditionally in sheep farms in Babel. So, the results obtained from FECRT and EHA indicate presence of different degree of resistance to Benzimedazole in gastrointestinal nematodes of sheep in all studied farms in Babel-Iraq. This compare well with many published reports about the resistance of nematodes to albendazole and levamisol in sheep (16,17,18).The relatively high efficacy of ivermectin with suspected resistance in only one farm provide warning about beginning of resistance to macrocyclic lactone members, which may be due to increased usage of ivermectin as a safe anti nematode drug in last few years. In other hand the resistance to macrocyclic lactone was recorded in many studies (19, 15, and 20).The combination results obtained by both FECRT and EHA showed that the both techniques should be used together to evaluate the resistance to benzimedazole group, and this fact in agreement with (21 and 22). In this study the *Haemonchus* predominated in larval culture of feces in all studied farm, these results supports general experience of many authors whose explain the ability of *Haemonchus* to develop resistance against most familiar anthelmintics(15 ,17).The comparative study of CT solution and thiabendazle is a simple attempt to encourage the research about herbal antiphrastic activity, the results showed that the minimal concentration results 100% EHA was 0.5 µg/ml and this indicate presence of anthelmintics resistance as mentioned above, while efficacy of CT have revealed relatively high dose *in vitro* anthelmintic activity against egg with poor effect on adult worm ,both these result are compatible with finding of others (12 and 23).the authors (15) explain the difference between relatively poor *in vitro* result with the good information obtained from herbal healers to the considerable variation in conditions encountered the *in vivo* like metabolic biotransformation ,interaction with feed and absorption. Therefore, these results should be ascertained by *in vivo* evaluation.The some extent significant result of tannin –rich solution on eggs of *Haemonchus* confirm the hypothesis of direct anthelmintics activity associated with extracts of tanninferous plants (23),and in agreement with findings of (24) in goats. We

recommended that further basic information required to better understanding of role of individual competent of tannin as well as demonstrated the possible anti-nutritional consequences.

References

1. Vlassoff, A.; Leathwick, D.M. and Heath, A.C.G. (2001) .The Epidemiology of Nematode Infections of Sheep. New Zealand Vet. J. 49 (6):213-221.
2. Sykes A.R. and Coop R.L. (2001). Interaction between nutrition and gastrointestinal parasitism in sheep. New Z Vet J, 49 (6):222-226.
3. Poppi, D.P.; Sykes, A.R. and Dynes, R.A. (1990). Thee of endoparasitism on host nutrition. Por.N.Z. Soc.Anim. Prod., 50:237-243.
4. Pedreira,E., J.; Feijoo, A.; Paz, A.; Sanchez-Andrade, R.; Suarez, J.L.; Diaz, P.; Lopez, C.; Diez-Banos, P.and Morrondo, P. (2003). Digestive parasitosis in ovine from Galicia (Spain) 11^{the} International Congress of Mediterranean Federation for Health and Production of Ruminants. Olbia (Italy), 22-25 may 2003.
5. Robertson , H.A.; Niezen , J.H.; Waghorn G.C.; Charleston ,W.A. and Ginlog ,M. (1995). The effect of six herbig on live weight gain wool growth and fecal egg count of parasitized ewe lambs . PRO. N.Z. Soc. Anim. Pro. 55: 199-201.
6. Coop, R.L. and Holmes, P.H. (1996). Nutrition and parasite interaction .International Journal of Parasitology .26:951-962.
7. Waghorn, G.C.;Shelton, I.D.; McNabb , W.C.; McCutcheon, S.N. (1994). Effect of condensed tannins in *Latus Pedunculatus* on Its nutritive value for Sheep. J. Agric .Sci. Camb., 123: 109-119.
8. Jones, G.A.; McAllister, T.A.; Muir, A.D. and Cheng, K.J.(1994). Effect of sainfoin (*Onobrychis viciifolia*) condensed tannins on growth and proteolyusis by four strains of ruminal bacteria .Appl. Environ. Microbiol.60:1374-1378.
9. Bae, H.D.; McAllister, T. A.; Yanke, J.; Cheng, K.J. and Muir, A.D. (1993). Effect of condensed tannins on endo glucose activity and filter raper digestion by Fibro Bacter Succinogenes . 585. App. Enviro. Microbial, 59:2132-2138.
10. Junes, W.T.; Broadhurst, R.B. and Lytteton, Y.W. (1976). The condensed tannins of atterns legums species. Phytochemistry . 15:1407-1409.
11. Colse, G. C.; Bauer, C.; Borgs, F. H. M.; Geerts, S.; Klei, T. R.; Taylor, M. A. (1992). Methods for the detection of anthelmintics resistances in nematodes of veterinary importance. Veterinary Parasitology, 44: 35-44.
12. Adama K.; Belem, A. M.; Tamboura, H.; Traore, A. and Sawadogo, L. (2009). *In Vitro* anthelmintic effect of two medicinal plants (*Anogeissus leiocarpus* and *Daniellia oliveri*) on *Haemonchus contortus*, an abosomal nematode of sheep in Burkina Faso. African Journal of Biotechnology. 8 (18):4690-4695.
13. Anonymous (1984). Ministry of Agriculture, Fisheries and Food Agriculture Development and Advisory Service Manual of Veterinary Parasitology Techniques, (HMSO), London.
14. Jackson, F.; Jackson, E. R. and Coop, J. (2002). Egg hatch assay for determination of resistance to nematodes to benzimidazole anthelmintics. In: Practical exercise

in Parasitology. (Halton, D. W.; Behnke,J ;Marshall, M. I. Eds.). Cambridge University press. UK. Pp, 313-318.
15. Eguale, T.; Tilahun, G. Gidey, M. and Mekonnen, Y. (2006). *In Vitro* anthemintic activities of four atterns medicinal plants against *Haemonchus contortus*. Phamacology, 3: 153-165.
16. Leathwick, D. M. (2001). Anthelmintic resistance in New Zealand. New Zealand Veterinary Journal 49 (6): 227-235.
17. Swarnkar, C. P. D.; Singh, F. A. Khan, P. S. K. and Bhagwan, K. (1999).Multiple anthelmintic resistances in *Haemonchusa contortus* of sheep. Ind. J. Anim. Sci., 63:547-549.
18. McKenna, P. B. (1994). The occurrence of anthelmintic-resistant sheep nematodes in the southern north-island of New-Zealand. New Zealand Veterinary Journal, 42(4): 151-152.
19. Hughes, P. L.and McKenna, P. B. (2005). A survey of the prevalence of emerging Macrocyclic Lactone Resistance and of Benzimidazole Resistance in Sheep Nematodes in the lower north island of New Zealand. New Zealand Veterinary Journal, 53 (1): 87-90.
20. Maciel, S.; Gimemez, A. M.; Gaona, C.; Waller, P. J. and Hansen, J. W. (1996). The prevalence of anthelmintic resistance in nematodes parasites of sheep in southern Latin America: Paraguay. Veterinary Parasitology, 62: 207-212.
21. Martin, P. J.; Anderson, N. and Jarret, R. G. (1989). Detecting benzimidazole resistance with fecal egg count reduction tests and *in vitro* assays. Australian Veterinary Journal, 8: 236-240.
22. Álvarez, M. A.; Mainar-JAIME, R.C.; Perrez-Garcia, J. and Rojo, F. A. (2002). A review of the methods for the detection for anthelmintic resistance. Revista Ibérica de Parasitología, 62 (1-2): 51-59.
23. Hoste, H.; Leveque, H. and Dorchies, P. (2001). Comparison of nematode infections of the gastrointestinal tract in angora and dairy goats in a rangeland environment: relations with the feeding behavior. Vet. Parasitolology, 101: 127-135.
24. Osoro, K. ;Benito-Peña ,A. ;Frutos , P. ;García ,U.; Ortega-Mora, L.M.; Celaya, R. and Ferre, I.(2007).The effect of heather supplementation on fecal egg counts of gastrointestinal nematodes and live weight changes in cashmere and local celtiberic goats on Pasture. Small Rumin. Res., 67: 184-191.

حدوث مقاومة ديدان المعدة والامعاء في الاغنام في بابل مع دراسة فعالية مركز العفصيك على الاجناس المقاومة

حيدر محمد الرماحي
كلية الطب البيطري/جامعة بابل

الخلاصة

اجريت الدراسة في ثلاثة قطعان اغنام محافظة بابل ,حيث تم تقييم مقاومة الديدان الاسطوانية ضد البندازول ,الليفاميزول والايفرمكتين بالاضافة الى تقييم فعالية محلول العفصيك التجاري ضد الاجناس المقاومة.اعتمادا على حد الثقة الادنى لنسبة اخفاض عدد البيوض في البراز FECR% الاقل من 95%,اظهرت النتائج ان حيوانات القطيع الاول المعالجة بالليفاميزول والايفرمكتين اظهرت مقاومة ضعيفة لهما بينما اعتبرت الحيوانات المعالجة بالبندازول مقاومة له.اما حيوانات القطيع الثاني والمعالجة بالليفاميزول والبندازول فاظهرت مقاومة مشكوكة بينما لم تسجل نسبة انخفاض عدد بيوض البراز اقل من 95% في الحيوانات المعالجة بالايفرمكتين. وعلى نفس المنوال, اظهرت حيوانات القطيع الثالث المعالجة بالبندازول و الليفاميزول

مقاومة ومقاومة ضعيفة على التوالي بينما لم تسجل اي مقاومة في الحيوانات المعالجة بالايفرمكتين واظهرت النتائج ان الجمع بين تقنيات الفحص في الزجاج وتقنيات الفحص في الجسم ضرورية لتقييم مقاومة الديدان الاسطوانية ضد مجموعة البنزاميدازول. كانت الاجناس الاكثر شيوعا والمشخصة من زرع براز الحيوانات قبل العلاج هي *Haemonchus* و*Trichostrongylus*, *Cooperia* بينما كان *Haemonchus* هو الجنس الوحيد المشخص من زرع البراز بعد العلاج.

ان مقارنة فعالية مركز العفصيك والثايوبندازول في الزجاج اظهرت ان كلاهما احدث فرق معنوي في تثبيط فقس بيوض الديدان وكان التركيز الاعلى المسبب لثبيط فقس الديدان 100 % هو 0.5 ملغم /مل و 2 ملغم /مل للثايوبندازول ومركز العفيك على التوالي,كما اظهر اختبار حركة الطفيليات البالغة ان الثايوبندازول قتل كل الديدان بتركيز 0.25 ملغم /مل, بينما لم يؤثر مركز العفصيك على الديدان حتى في اعلى تراكيزه المستخدمة في الاختبار.