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COMPARATIVE EFFICACY OF ALBENDAZOLE, LEVAMISOLE AND IVERMECTIN TREATMENT AGAINST GASTRO-INTESTINAL NEMATODES IN EWES DURING PREGNANCY AND THEIR EFFECT UPON PRODUCTIVE EFFICIENCY

(With 4 Tables)

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مقارنة كفاءة الالبندازول والليفاميزول والايفرميكتين لعلاج الديدان المعوية في النعاج العشار وتأثيرهم على الكفاءه الانتاجية

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أجريست همذه الدراسة لمقارنة كفاءة عقار الالبندازول والليفاميزول والايفرميكتين فيعلاج المنعاج العشمار المصابة بالديدان المعوية. قسم العدد الكلى للنعاج العشار (٢٥ نعجة) المي خمســة مجاميع متساوية. المجموعة الاولى استخدمت كمجموعة ضابطة بدون علاج بينما عولجت المجموعة الثانية بجرعتين من الالبندازول بين كل منهما ثلاثة أسابيع. والمجموعة الثالثة عولجت بجرعتين من الليفاميزول بين كل منهما ثلاثة أسابيع. أما المجموعة الرابعة فقد عولجت أيضا بجرعتين بين كل منهما ثلاثة أسابيع، الجرعة الآولي بالالبندازول والثانية بالليفاميزول. عولجت المجموعة الخامسة بجرعة واحدّة من الايفرميكتين. أخذت عينات بر از من جميع النعاج قبل العلاج وكل أسبوع بعد العلاج طوال فنرة التجربة. وجد أن التأثير على الديـــدان المعويـــة يكـــون أقـــوى بـــالعلاج بالالبــندازول والليفاميزول معا وأيضا العلاج بالايفرميكتين مقارنة بالعلاجات الأخرى. وبمقارنة صورة الدم وبعض التحاليل البيوكيميائية لكل المجاميع بعد ستون يوما من العلاج، وجد أن اصابة المجموعة الضابطة بالديدان الاسطوانية لها تأثيرا سلبيا حيث انها سببت انخفاضا معنويا في نسبة الهيموجلوبين في الدم، والبروتين الكلى والكالسيوم والفوسفور فى مصل الدم مع زيادة تركيز نسبة الكوليستيرول فى مصل الدم وزيادة عدد كرات الدم البيضاء والايزينوفيل. أدى العلاج بالالبندازول الى تحسن هذه القياسات بمعدل محدود، أما الكوليستيرول فلم تتأثر نسبته بعد العلاج. بينما أدت العلاجات بالعقاقير المختلفة الأخرى إلى زيادة هذه القياسات وتحسنها معنويا. أثرت اصابة المجموعة الضابطة بالديدان المعويه سلبيا على معدل أوزان الحملان الناتجة منها مقابل الزيادة في معدل الأوزان في الحملان الناتجة من النعاج المعالجة. وبمقارنة متوسطات أوزان الحملان للأمهات المعالجة بمختلف العقارات المضادة للديدان المعوية لوحظ عدم وجود تأثير

معنوى للعلاج بالالبندازول مع وجود تأثيرا معنويا عاليا على وزن الحملان في بقية المجاميع المعالجة بالعقارات المختلفة الأخرى.

SUMMARY

The anthelmintic efficacies of Albendazole, Levamisole and ivermectin treatment were studied on twenty five pregnant ewes naturally infected with gastrointestinal nematodes (GIN). The pregnant ewes were divided into five equal groups. The first group was left as untreated control, ewes of the second group treated with Albendazole on two recommended doses with three weeks interval. The ewes of the third group were treated with Levamisole on two recommended doses with three weeks interval. The fourth group were treated with Albendazole, and after three weeks they were given a second dose with Levamisole. The fifth group were treated with one recommended dose of ivermectin. Feacal samples were collected from all ewes before treatment and every seven days posttreatment throughout the study. The anthelmintic activity of combination of Albendazole with Levamisole treatment and ivermectin were greater than the other used drugs. Group comparison by ANOVA test for the measured haematological and biochemical parameters sixty days posttreatment, showed that the untreated ewes had significantly (< 0.05) lower haemoglobin concentration, serum total protein, albumin, calcium and phosphorus concentrations, with increased serum cholesterol concentration, total leukocytic count and eosinophilia. In Albendazole treated group, these parameters improved moderately except serum cholesterol concentration compared with the control group. In Levamisole, combination of Albendazole with Levamisole and ivermectin treatments, these parameters were improved and reached to the normal levels. GIN exerted a negative impact on the lamb live weights from infested ewes, as seen by improving these weights following anthelmintic treatment of ewes. Group comparison of the means of the lamb live weights between the different groups revealed non-significant effect of Albendazole and significant (< 0.05) higher effect of Levamisole, combination of Albendazole and Levamisole treatment and ivermectin treatment.

Key word: Albendazole, Levamisole, Ivermectin, nematodes, pregnancy, productive efficiency

INTRODUCTION

Parasitic gastroenteritis is a common and major problem in small ruminants and responsible for heavy economic losses (Schalm, 1979). Mixed infestations with multiple genera (Ascaris, Haemonchus, Ostertagia, Teladorsagia, Trichostrongylus and Cooperia) occur and usually are responsible for disease conditions (Lora Rickard, 2001). The economic importance of gastrointestinal nematodes (GIN) is through decreased weight gains, impaired wool, milk production and poor reproductive performance (Holmes, 1985). In Egypt, outbreaks of parasitic gastroenteritis in sheep have been reported by many authors (El-Degheidy 1981; Mahmoud 1980 and Abdel all, 1990). Periodical faecal examination of breeding ewes and female goats indicated that faecal egg counts are tended to increase rapidly in the periparturient period with consequent production losses in these animals (Reid and Armour, 1975; Swarnkar et al., 1998 and Dalal, 2000). In addition, even low level of infestation can adversely affect the ewe's metabolism (Mackay 1980). Anthelmintic treatment of ewes during pregnancy virtually eliminates ewe parasites and lead to significantly higher lamb weights (Familton et al., 1995). Anthelmintics used for elimination of GIN have varying degrees of success (Bogan and Marriner, 1987). Apparently, the increased use of anthlmintics may lead to parasitic resistance (Condor and Campell, 1995). Albendazole, Levamisole and ivermectin are broad spectrum anthlmintics with different mode of actions. Albendazole is \(\beta\)-tubulin binding anthelmintic, Levamisole is nicotinic agonists anthlmintics drug and ivermectin is glutamate- gated chloride receptor potentiator (GIUCL potentiator) drug (Martin, 1997).

Therefore, the current study aimed to compare the efficacy of these anthlmintics against gastrointestinal nematodes of ewes during pregnancy and their effects on production.

MATERIAL and METHODS

1- Animals:

The present study was carried out at Animal Reproduction Research Institute, Sheep Farm, and it was conducted on twenty five adult Barky ewes (n= 25) of nearly the same age and proved to be naturally infested with gastrointestinal nematodes through laboratory examination of faecal samples. Animals were fed a balanced ration supporting the nutrient requirements of ewes. These ewes were housed with three rams of proven fertility, for one month for insemination. Pregnancy was confirmed by Ultrasonograph.

2-Treatment trials:

Animals were divided into 5 groups (n=5 each). Ewes in group I served as untreated control (positive control). Ewes in group II were treated with oral Albendazole (Albendazole, product of Pharma Swede Egypt, each 1ml contain 25 mg) at 2ml/10 kg B.Wt. Ewes of group III subcutaneous injection of Levamisole (Levamisole received hydrochloride, product of chemical industries development Pharmacia, 7.5% conc.) at 4 ml per animal weighing 40-80 kg. In group II and III. the drugs were given twice during pregnancy; the first dose was given one month after removal of rams (Yolande Bishop, 1998), and the second dose given three weeks apart. Re-administration of the drugs in both groups aimed to increase the efficiency of the drugs and to eliminate the immature stages which reach maturity 2-3 weeks posthatching for most genera of GIN (Lora Rickard, 2001). Ewes of group IV was given Albendazole one month after removal of rams with the same dose as in group II, after three weeks, the ewes received subcutaneous injection of Levamisole with the same dose as in group III as a second dose. Ewes in group V received subcutaneous injection of ivermectin (Bomectin^R, product of BoMAC Laboratories, 1% W/V) at 1ml/50kg B.Wt

3- Faecal examination:

Faecal samples were collected from rectum of each ewe before treatment and each week post-treatment till the end of the trial, and examined for the presence of nematode eggs by saturation concentration floatation technique using saturated sodium chloride solution (Soulsby, 1982). Degree of infestation was performed by egg counting using the McMaster technique according to Urquhart et al., (1988). Faecal culture for identification of the infective larvae was applied according to Georgi et al., (1985).

4- Sampling:

Blood samples were obtained from each ewe sixty days after treatment. About 2ml of anticoagulated blood were collected in sterile vials containing disodium-EDTA for determination of blood picture. And about 10 ml of blood was collected from each animaL in clean sterile vacutainer AND were allowed to stand overnight at 10 0 C and centrifuged (2,000 r.p.m for 20 minutes) to obtain sera. Serum was stored at -20 0 C untill biochemical analysis.

5- Haematological and biochemical analysis:

Anticoagulated blood samples were used for estimation of haemoglobin concentration calorimetrically according to Jain (2000) using Drabkin's reagent. Total leukocytic count was applied according to Wintrobe (1967) and the differential leukocytic count was performed according to Feldman *et al.*, (2000). Blood serum samples from all ewes were analyzed for total protein, albumin, cholesterol, calcium and phosphorus using standard diagnostic kits according to the method of Kato, 1960; Rodkey, 1964; Watson, 1960; Gindler and King, 1972 and El-Merzabani *et al.*, 1977 respectively.

6- Live weight evaluation:

All newly born lambs were weighed at birth and at 15, 30, 60, 90 and 120 days after birth for determination of the growth rate of lambs.

7- Statistical analysis:

The data were analyzed using Statistical Analysis System (SAS, 1993). ANOVA Procedures were used to detect the difference between different groups. The data represented as mean \pm standard error.

RESULTS

In the present study, examination of faecal samples of ewes showed that they were naturally infected with different species of GIN. Faecal culture revealed that, the commonest gastrointestinal nematodes were Haemonchus sp., Trichostrongylus sp., Bunostomum sp., Chapertia sp., Strongyloioedes papillosus, and Trichuris sp. Samples of the first group (untreated and positive control) showed that, the faecal egg count per gram (FEC) was (100-892) and increased throughout the pregnancy to (400-1300) as shown in table (1). In ewes treated with anthelmintic drugs, the FEC in the third, fourth and fifth groups, were reduced to nil by seven day after the re-administration of the drugs, while in Albendazole treated group (G II) the reduction percent was 80% which increased to 95% after re-administration. In addition, in Levamisole treated group (G III) the reduction percent was 95% which increased to 100% after re-administration. Re-infestation was found in the ewes of the second, third and fourth groups after treatment by (50 \pm 1.89), (89 \pm 23.76) and (96 \pm 12.86) days respectively. In the ivermeetin group, no re-infection was evident till the end of the trial (120 days).

The effect of Anthelmintics given to infected pregnant ewes on some blood parameters after sixty days post-treatment were illustrated in table (2). Ivermectin treatment significantly (< 0.05) increased the haemoglobin concentration than the other treatments. The combination

of Levamisole and Albendazole treatment significantly (< 0.05) decrease the total leukocytic count than the other treatments. The mean values of lymphocytes, neutrophils, monocytes and eosinophils returned to the normal values after treating the ewes with the Anthelmintics. Table (3) illustrated the effect of different anthlmintics on some biochemical parameters sixty days post-treatment. Both Levamisole and ivermectin treatments significantly (< 0.05) increased the serum total Serum concentration than the other groups. concentration significantly (< 0.05) increased after treating the ewes with the anthlmintics in all groups compared with the control group. Serum cholesterol concentration returned to the normal values after treating the ewes in all groups compared with the control group except with Albendazole and ivermectin groups. Levamisole significantly (< 0.05) increased serum calcium and phosphorus concentration than the other groups.

The means of the lamb live weights on birth day, 15, 30, 60, 90 and 120 days were shown in table (4). Group comparison by the ANOVA test revealed non-significant (< 0.05) effect of Albendazole treatment compared with the control group, whereas Levamisole was significantly (< 0.05) higher in the lamb weights at birth, 15, 30, and 120 days than the control group. Similarly the means of lamb weights at birth, 15, and 120 days in GIV and GV were very significantly (< 0.05) higher than the other groups.

DISCUSSION

In the present study, GIN infestation in untreated ewes, showed low concentration of serum haemoglobin, total protein, albumin, calcium and phosphorus. On the other hand, it showed increase concentration of serum cholesterol and increased the number of total leukocytic count associated with eosinophilia. Same results have been reported by Sykes, (1983); Swarup and Pachauri, (1986) and Dalal, (2000). These abnormal levels and subsequent stressful wasting syndromes in the untreated ewes may be the cause of decreasing the means of lamb live weights on birth days and thereafter till weaning. Tekelye *et al.*, (1992) and Mukasa *et al.*, (1993), reported similar observations.

Anthelmintic treatments have wide differences in efficacies on GIN which often reflective of their differential spectra of anthelmintic activities, pharmacokinetics and the degree of synchronization of treatment programme with the life cycle of the target helminthes parasites (Brunsdon, 1980). From the data presented in table (1)

Albendazole treatment showed reduction of faecal egg count by 80%, which was in agreement with Selim, 1996 and William and Broussad, (1995). This result may be due to un-wise increased use of this compound and appearance of anthelmintic resistance (Prichard, 1994).

Significant greater effectiveness of Albendazole on GIN was observed by re-administration of the drug after three weeks (the reduction rate was 95%) which agree with Rew and Knight, 1980 and Anee and Terry, 1999. the significant shorter time elapsed from Albendazole treatment till re-infection (50.5 ± 1.89 days) may be due to it's pharmacokinetic characteristics " it's greater solubility, rapid absorbance, oxidation in the liver and excretion in urine) which may lead to reduce the persistent effect of it's rumen reservoir (Steel and Hennessy, 1999).

Concentration concerning the measured haematological and biochemical parameters in ewes after sixty days of treatment with Albendazole compared with the other treated groups were moderate with non significant difference in serum cholesterol concentration between Albendazole treated vs. untreated control group.

There were non-significant differences between the live weights of lambs on birth day and thereafter in Albendazole treated group vs. untreated control group which coincided with those previously obtained by Taylor *et al.*, (1997). These results could be due to the incomplete elimination of nematode eggs and its appearance again after Albendazole treatment than the other treated groups.

Levamisole treatment reduced the faecal egg count by 95% which increased to 100% by re-administration of the drug. The drug readministration three weeks later to eliminate the immature stages and limits the level of pasture contamination (Okewole *et al.*, 1982), particularly as Levamisole is less effective against the immature forms of GIN (Donald, 1999). The measured haematological and biochemical parameters in ewes after sixty days of Levamisole treatment improved and reached to the normal levels with significant increase of serum total protein, calcium and phosphorus. Similar results observed by previously Ali *et al.*, (1994).

Concerning the fourth group, the treatment of ewes with combination of Albendazole and Levamisole, significant decreased the FEC and increased the time after treatment till re-infection (96 \pm 12.86 days) compared with the second and third groups. The same results were recorded by Froylan *et al.*, (2001). The synergistic effect of both drugs may be due to the fact that each one of them has specific mode of action

and combining them together increase the efficacy of the combination (Martin, 1997). Leukocytosis associated with eosinophilia due to the parasitic infestation significantly decreased after treatment with combination of Albendazole with Levamisole than the other treated groups. This indicated the strong effect of both drugs when combined together. Moreover, the other measured haematological and biochemical parameters significant improvement after treatment.

Ivermectin treatment reduced the output of eggs in the faeces of the ewes to nil (reduction rate was 100%) and the period of suppressed egg output was extended till the end of the trial. The same results were observed by Mackellar et al., (1988); Mackellar and Marriner, (1987); Borgsteede, (1993) and Williams and Broussard, (1995). The more persistent anthelmintic effect of ivermectin given subcutaneously was probably due to its extended half life in the plasma of treated ewes (McKellar and Marriener, 1987). Haematological and biochemical analysis in ivermectin treated group, showed significant increase in haemoglobin and serum total protein concentration, while there was moderate increase in serum calcium and phosphorus concentration compared with the other treated groups, this result was in agreement with Selim, (1996) and Kumar and Suryanarayana, (1995). Serum cholesterol concentration did not return to the normal levels after ivermectin treatment, Hany et al., (1995) showed hypercholesterolemia in rams treated with ivermectin and suggested that, this effect might be due to the liver dysfunction caused by ivermectin.

The relatively higher lamb live weights of ewes treated with Levamisole, combination of Albendazole with Levamisole, and ivermectin compared with the infested control ewes provide proofs of efficacy for the drugs used. Similar observations for higher lamb weights from ewes free from harmful nematodes were previously reported by Ramadan and Khodre, (1982); Mukasa et al., 1991 and 1993. In conclusion, the degree of anthelmintic elimination of nematode infestation especially shortly after coitus (Hope Cawdery, 1979) enhanced productivity of the ewes appeared in increased lamb weight.

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Table 1

Effect of different Anthelmintics on faecal egg count (FEC) of infested pregnant ewes with GIN

Animal group	Drug used	1 st Dose	FEC (range) /gm				D 1	Time elapsed from
			Before treatment	After treatment	Reduction %	2 nd Dose	Reductio n%	treatment till re- infection (days)
GI		ı	100 -890	400 -1300	0			
GII	Albendazole	2ml /10kg BW orally	160-8100	3 -150	80	2ml/10kg BW orally	95	50.5 ± 1.98ª
GIII	Levamisole	4ml /40-80 kg BW S/C	150-4750	3-50	95	4ml /40-80 kg BW S/C	100	89 ± 23.76 ^b
GIV	Albendazole & Levamisole	2ml /10kg BW orally	350-3900	4 -200	80	4ml /40-80 kg BW S/C	100	96 ± 12.86 ^b
GV	Ivermectin	1ml/50kg BW	200 -7750	0	100		100	0

a, b and c superscripts with different letters within a column are significantly different (< 0.05) Data were presented as mean \pm SE

Table 2
Haematological Parameters of Pregnant Ewes sixty Days Post-treatment With
Different Anthelmintic Drugs in Comparison With Untreated Control Ewes

Measured parameters	GI	GII	GIII	GIV	GV
Hb (g/dl)	8 ± 0.35 *	10.03±0.44 ^b	9.88 ± 0.75 b	10.37±0.46 bc	11.72±0.9 °
TLC (x10 ³)	18.62±0.8 ª	14.09±2.02 b	12.93±2.035 bc	11.95±0.99 °	12.97±1.69bc
Lymphocyte %	59±3.25 *	55.75±4.84 *	55.90±4.05 *	47.62±4.23 b	51.15±6.91 b
Neutrophil%	30±1.7 *	36.45±5.13 ^{bc}	34.1±2.15 ⁶	43.75±3.83 °	43±7.22 °
Monocyte %	3±0.45*	3.75±1.12 ^{ab}	3.85±1.03 ^{ab}	5.25±1.77 b	2.65±0.63 *
Basophile %	0	0.062±0	0	0	0.001±0
Eosinophil%	8±0.75	3.73±0.95 b	4.5±1.95 b	4.07±1.53 b	3±0.73 b

a, b and c subscripts with different litters within a column are significantly different (< 0.05) Data were presented as mean \pm SE

Table 3

Biochemical Analysis of Serum Parameters of Pregnant Ewes Sixty days Posttreatment with Different Anthelmintic Drugs in Comparison with Untreated
Control Ewes

	Gl	GII	GIII	GIV	GV
Total proteins (g/dl)	5.26 ± 0.31^a	6.47± 0.31 b	7.85 ± 0.86 °	7.4 3± 0.42 ^{cb}	7.54 ± 0.93 °
Albumin (g/dl)	2.97 ± 0.26°	3.73 ± 0.19 b	3.70 ± 0.33 b	4.11± 0.38 b	4.17 ± 0.36 ^b
Cholesterol (mg/dl)	96.47± 4.7 °	86.92 ± 6.35 *	81.27± 9.57 b	80.63 ± 10.63 b	100 ± 10.7 °
Calcium (mg/dl)	6.1 ± 0.04^{a}	7.60 ± 0.58^{-6}	11.65 ± 1.48 °	10.4 ± 1.53 °	8.60 ± 0.85 b
Phosphorus (mg/dl)	4.2 ± 0.32 *	5.58 ± 0.67 b	7.04 ± 0.7 °	6.27± 0.78 bc	5.9 ± 0.66 b

A, b and c subscripts with different litters within a column are significantly different (< 0.05) Data were presented as mean \pm SE

Table 4
Effect of Anthelmintic Drugs given to Infested Pregnant Ewes on Lambs Live
Weight

BW of lambs	GI	GH	GIII	GIV	GV	
At birth	3 ± 0.29^a	3.25 ± 0.16^{4}	3.8 ± 0.13^{b}	$3.9 \pm 0.08c$	4.8 ± 1.4^{c}	
after 15 days	6.3 ± 0.5^{a}	7.1 ± 0.8^{8}	7.25 ± 1.25^{b}	8.3 ± 0.17^{c}	$7.75 \pm 0.9^{\circ}$	
after30 days	9.6 ± 0.33^{4}	10.25 ± 1^{8}	11.75 ± 1.13^{b}	$14.5 \pm 0.33^{\circ}$	12.12 ± 0.25^{b}	
after 60days	14.3 ±0.66*	14.83 ± 0.4^{a}	14.8 ± 0.25^{a}	$19.5 \pm 0.86^{\circ}$	$17.5 \pm 0.5^{\circ}$	
after 90days	19.6 ± 2.3^a	19.16 ± 2.3^a	20 ± 1.25^{a}	23.3 ± 0.66°	22 ± 1.7^{b}	
after 120 days	21.5 ± 1.33^{a}	22.37 ± 0.23^a	24 ± 0.92^{b}	$27.33 \pm 0.66^{\circ}$	$28.5 \pm 1.5^{\circ}$	

A, b and c subscripts with different litters within a column are significantly different (< 0.05) Data were presented as mean \pm SE