RESISTANCE OF *Fasciola hepatica* TO TRICLABENDAZOLE, CLOSANTEL, AND RAFOXANIDE IN A SHEEP FARM IN SHARAZOR DISTRICT, KURDISTAN-IRAQ

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ABSTRACT

Fasciolosis caused by *Fasciola hepatica*has long been one of the most important helminthic infections of livestock in Iraq. The control of this parasite is based on the use of anthelmintic agents, mainly triclabendazole and closantel. Sheep on a farm in Tut Aghach village, Sulaymaniyah, Iraq died from liver fluke disease in spite of previous treatment with triclabendazole. Thisstudy was conducted to investigate the effect of three commercially available anthelmintic agents – triclabendazole, closantel, and rafoxanide – against natural liver fluke infection in sheep. Fecal egg count reduction test (FECRT) was used to determine the efficacy of the drugs.Treatment with triclabendazole resulted in 73.9% reduction in fecal egg count (FEC) with the lower confidence interval of 61.0%. Closantel and rafoxanide reduced the FEC in the treated groups by 70.1% and 70.2%, respectively. This study confirms the resistance to closantel, rafoxanide, and triclabendazole of the *F. hepatica* from a sheep farm in Sharazor district, Sulaymaniyah, Iraq. A study of more farms from endemic areas in Iraq is required to evaluate the burden of resistance development against the common anthelmintic agents.

INTRODUCTION

Fasciola hepatica is a parasitic trematode found in temperate and high-altitude tropical areas, it is commonly known as liver fluke. This parasite causes liver disease (fasciolosis) in a wide range of host species. Although ruminants are most commonly affected(1), fasciolosis has been described in a range of mammals including horses(2)and camels(3). The parasite is transmitted by a snail as an intermediate host (4).

The most effective and widely used anthelmintic for the treatment of fasciolosis in animals is triclabendazole(5), which is highly effective against both mature and immature stages of liver flukes (6, 7).Triclabendazole was introduced in the 1980s for the therapy of liver fluke infections in livestock and it has become the drug of choice for the treatment of human fasciolosis (8).Closantel and rafoxanide are two salicylanilides which have also been used against liver flukes in livestock. However, these drugs are not as effective against immature flukes as triclabendazole(9).

Fasciolosis has been previously documented from different parts of Iraq (10, 11). However, no studieshave been conducted, and no evidence of resistance of *F. hepatica* against triclabendazole and closantel has been reported yet. Asheepfarm in Sharazor district, Sulaymaniyah city, Kurdistan region was previously treated with triclabendazole but the therapy failed to clearthe infection from the animals. Consequently, many sheep died from acute fasciolosis. Hence, this study was conducted to investigate the susceptibility of *F. hepatica* against the commercially available triclabendazole, rafoxanide, and closantel under field conditions.

MATERIALS AND METHODS

Farm and animals

The study was conducted in a sheep farm in Tut Aghachvillage that is located in the northern part of Sharazor district, 43 km southeast ofSulaymaniyah city (Figure 1).The farm had a long history of liver fluke infection and was regularly treated with triclabendazole, biannually. The sheep were grazed on pastures where the intermediate host *Lymnaeatrun catula* was found. The farm had several cases of sudden death among the sheep, without noticeable clinical symptoms. Upon post mortem examination, it was evident that the mortality was due to acute fasciolosis. The owner stated that the flock was treated with triclabendazole less than two months ago.

Ninety sheep of different ages were randomly selected and divided into three groups, each containing 30 animals.The groups were treated with 5 mg/kg closantel (CLOSANTEL 10%, VAPCO, Jordan), 7.5 mg/kg rafoxanide (RAFOXIN, VAPCO, Jordan), and 10 mg/kg triclabendazole (TRICLASOL,Dosch, India), respectively. The dose was given based on the individual weights of the sheep.

Fecal samples were collected from the sheep before treatment and after 14 days and were tested for the number of liver fluke eggs by Fecal Egg Count Reduction Test (FECRT) (6).

Fecal Egg Count Reduction Test (FECRT)

A sedimentation method was used to determine the number of fluke eggs per gram (EPG) of feces. The sample was mixed well and 10 g of feces were weighed out and mixed

with water in a 500 ml beaker. The beaker was then topped up with water. Three sieves (38 µm, 150 µm, and 500 µm) were stacked with the smallest aperture at the bottom and largest at the top. The fecal water was slowly passed through the sieves followed by thorough washing with water until the water ran clear from the bottom sieve. The 500 µm sieve was removed and washing through the remaining two sieves was repeated. The 150 µm sieve was removed and the retentive on the surface of the 38 µm sieve was washed and the remaining contents were transferred into a 500 ml beaker. The beaker was topped up with water and left to stand for four minutes. The supernatant was poured off leaving approximately 100 ml of sediment and then the beaker was refilled with water and left to stand for four minutes. This process was repeated until the supernatant was clear. When clear, the supernatant was poured to 100 ml or less, if possible, without losing any sediment and the remaining content was transferred into a large square Petri dish. Four drops of methylene blue were added and the number of *F. hepatica* eggs counted using a dissecting microscope. The number of eggs per gram of feces(EPG)was calculated by dividing the total number of eggs by 10.

Statistical analysis

The Fecal egg count reduction (FECR) of the different drugs was calculated as $\frac{EPG\ day\ 0 - EPG\ day\ 14}{EPG\ day\ 0} \times 10$. The 95% confidence interval (CI) was calculated as $\{\frac{\text{EPG day 0 - EPG day 14}}{\text{EPG day 0}} \times 100\} \pm 1.96\ \sqrt{Y2}$, where Y2 is the variance of the FECR. *Fasciola hepatica* was considered resistant to a drug when the average FECR by the drug was <95% and the lower 95% CI was <90%(6). Efficacies of the different drugs were compared statistically using one-way analysis of variance, followed by post hoc (Duncan).

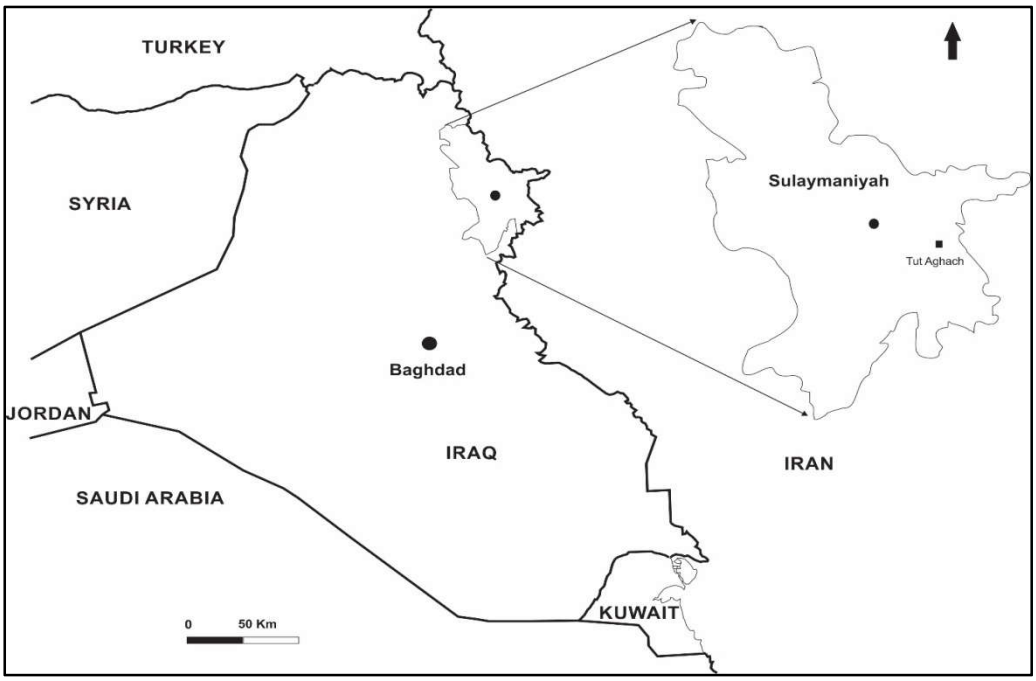

Figure 1 Administrative map of Sulaymaniyah, showing the location of Tut Aghach village.

RESULTS

The FECR was 100% in 11 sheep treated with triclabendazole. However, the fecal egg count increased after two weeks of treatment in two sheep (numbers 9 and 30, Table 1). The average FECR in the group of sheep treated with triclabendazole was 73.9% and the lower CI was 61.0%, which indicated that resistance has been developed by *F. hepatica* (Table 1, Figure 2).

Table 1 Results of the fecal egg count reduction (FECR) in the sheep after treatment with the different flukicidal drugs

Sheep no.	Triclabendazole			Rafoxanide			Closantel		
	FEC		FECR	FEC		FECR	FEC		FECR
	Day 0	Day 14		Day 0	Day 14		Day 0	Day 14	
1	650	240	63.1	350	0	100	400	240	40
2	600	0	100	600	320	46.7	700	160	77.1
3	1400	320	77.1	1900	400	78.9	300	80	73.3
4	1250	0	100	380	560	-47.4	400	240	40
5	950	320	66.3	850	250	70.6	240	320	-33.3
6	350	80	77.1	1300	80	93.8	400	0	100
7	100	0	100	1070	720	32.7	200	0	100
8	750	0	100	400	160	60	400	0	100
9	240	320	-33.3	900	450	50	600	560	6.7
10	600	80	86.7	500	80	84	450	160	64.4
11	720	0	100	850	280	67.1	650	80	87.7
12	600	240	60	500	200	60	550	0	100
13	750	80	89.3	1300	300	76.9	800	80	90
14	800	80	90	1300	640	50.8	600	80	86.7
15	550	240	56.4	700	300	57.1	450	80	82.2
16	525	500	4.8	500	0	100	450	0	100
17	1900	400	78.9	900	160	82.2	600	160	73.3
18	1100	0	100	650	0	100	650	160	75.4
19	620	80	87.1	1250	80	93.6	450	0	100
20	650	0	100	1400	240	82.9	1700	80	95.3
21	600	80	86.7	400	80	80	400	80	80
22	750	80	89.3	600	80	86.7	480	320	33.3
23	300	0	100	400	0	100	250	80	68
24	300	0	100	600	0	100	320	320	0
25	1200	0	100	1000	0	100	240	0	100
26	500	320	36	400	320	20	350	0	100
27	400	160	60	1350	160	88.1	550	320	41.8
28	420	240	42.9	1050	240	77.1	550	280	49.1
29	800	0	100	480	0	100	800	80	90
30	350	360	-2.9	400	360	10	1500	240	84

The minus signs indicate that the fecal egg count increased after treatment with the designated drug. FEC = fecal egg count.

Nine of the sheep treated with rafoxanide were free from *Fasciola* eggs after 14 days. The FECR of rafoxanide was 70.1% with the lower CI of 57.7%, while treatment with closantel resulted in 70.2% decrease in the fecal egg count with the lower CI of 57.4%. The fecal egg count increased in one animal (number 4) following therapy with rafoxanide.

The results indicated multidrug resistance of *F. hepatica* in the studied animals against all the tested antitrematodal agents. Comparison among the average FECR values of the three drugs revealed no statistical difference ($p \geq 0.05$), meaning that none of the tested drugs proved to be better than the rest.

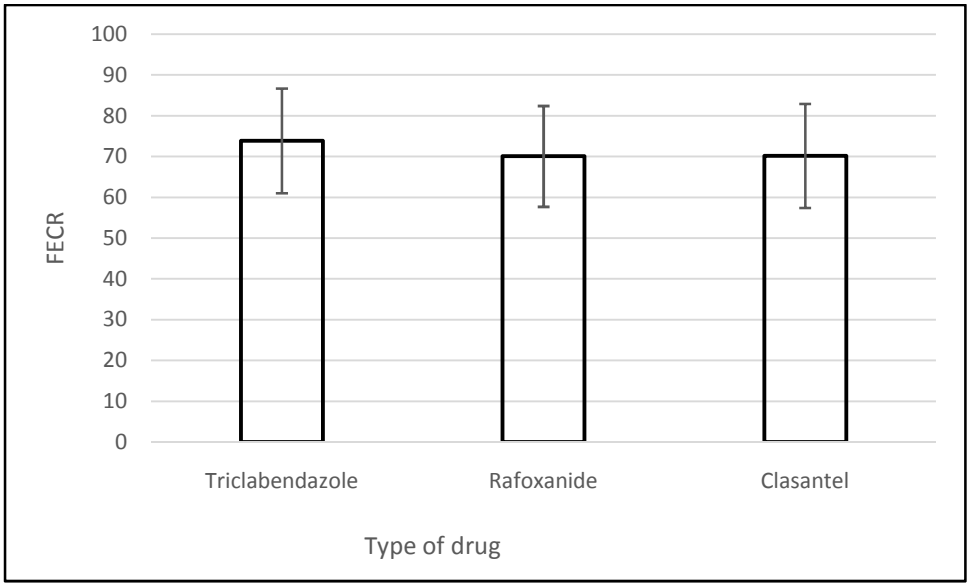

Figure 2 Average fecal egg count reduction (FECR) in the sheep (30 per group) treated with triclabendazole, rafoxanide, and closantel. Error bars indicate the 95% confidence interval.

DISCUSSION

Fasciolosis is one of the most common helminthic infections inIraq(12, 13).Anthelmintictherapy is considered one of the most important control measures since other ways of infection control such as elimination of the intermediate host and pasture management are considered costly and time-consuming.However, the results demonstrate the

emergence of multidrug-resistant *F. hepatica* in Tut Aghach village in Sharazoragainst triclabendazole, closantel, and rafoxanide.

Triclabendazole is one of the most widely used drugs by farmers against liver fluke infections in Sulaymaniyah for many years. It is one of the most important antitrematodal agents to treatfasciolosis since it is the only available drug that has the ability to eliminate early immature and adult *F. hepatica*at a dosage rate of 10 mg/kg (7, 14). According to the best of our knowledge, noprevious studyabout the failure of this drug to eliminate fasciolosis in Sulaymaniyah and Iraq is available. The results of this study showed the lack of efficacy of triclabendazole to treat fasciolosis in the infected sheep. This may lead to a serious problem to the livestock industry and human health in Sulaymaniyah. Most treatments given to the animals in this area are obtained by the livestock owners themselves without taking veterinary advice. Continuous use of triclabendazole by farmers would probably accelerate the pace at which resistance is developing.

Closantel and rafoxanide aresalicylanilideanthelminticsused against liver flukes. They act by uncoupling the oxidative phosphorylation in the parasite (15). Development of resistance against one drug usually results in the failure of the other drug as well since they share the same mechanism of action. The efficacies of closantel and rafoxanide in this study were 70.2% and 70.1%, with a lower CI of 57.4% and 57.7%, respectively. This indicates that other members of salicylanilides such as oxyclozanide and nitroxinil would most probably be ineffective against *F. hepatica* in the area. Resistance development against closantel, rafoxanide, and other members of the salicylanilides has not been reported previously in Iraq. Hence, this study is considered the first report about the emergence of multidrug-resistant *F. hepatica* in Sulaymaniyah governorate.

CONCLUSION

The present study confirms the emergence of multidrug-resistant *F. hepatica* to triclabendazole, closantel, and rafoxanide in a herd of sheep in Tut Aghach village, Sharazor district, Sulaymaniyah, Iraq.

مقاومة الدودة الكبدية*Fasciola hepatica* ضد ترايكلابيندازول، كلوزانتيل و رافوكسانايد بمزرعة في قضاء شهرزور في كردستان-العراق

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الخلاصة

لطالما كان مرض تعفن الكبد الذيتسببهدودة *Fasciola hepatica* من أهم الإصابات بالديدان الطفيلية في العراق. وتستند السيطرة على هذا الطفيليالى استخدام مضادات الديدان مثل ترايكلابيندازول وكلوزانتيل. توفيت مجموعةمن الخراف في مزرعة بقرية توت أغاج فى قضاء شهرزور في السليمانية بالعراق جراء مرض تعفن الكبد على الرغم من العلاج السابق باستخدام الترايكلابيندازول. وقد أجريت هذهالدراسة لتبيان تأثير ثلاثة ادوية متوفرة تجاريا – ترايكلابيندازول، كلوزانتيلورافوكسانايد–ضد عدوى تعفن الكبد الطبيعية في الأغنام. تم استخدام اختبار الحد من عدد البيض فى البراز (FECRT)لتحديد فعالية الادوية. نتج عن العلاج بالتريكلابيندازول انخفاض بنسبة ٧٣.٩ ٪ في تعداد البيض فى البراز(FEC)مع فاصل ثقة منخفض قدره ٦١.٠ ٪، كما خفض كلوزانتيل ورافوكساناید FEC في المجموعات المعالجة بنسبة ٧٠.١ ٪ و ٧٠.٢ ٪، على التوالي. تؤكد هذه الدراسة على مقاومةدودة *F. hepatica* من مزرعة غنم في شهرزور فيالسليمانية، العراق ضد كلوزانتيل ورافوكسانايد وتريكلابيندازول. هناك حاجة إلى دراسة المزيد من المزارع في المناطق الموبوءة في العراق لتقييم مديظهور المقاومة ضد الادويةالمبيدةللديدان الطفيلية.

REFERENCES

1.**Marcos L., Maco V., Samalvides F., Terashima A., Espinoza J.R., Gotuzzo E.**(2006). Risk factors for *Fasciola hepatica* infection in children: a case–control study. Trans R Soc Trop Med Hyg. 100:158-166.

2.**Arias M.S., Piñeiro P., Hillyer G.V., Francisco I., Cazapal-Monteiro C.F., Suárez J.L., *et al.***(2012). Enzyme-linked immunosorbent assays for the detection of equine antibodies specific to a recombinant *Fasciola hepatica* surface antigen in an endemic area. Parasitol Res.110:1001-1007.

3.**Eslami A., Ranjbar B.S.H., Eskandari A., Sedaghat R.** (2003). Study on the prevalence and pathology of *Fasciola* in camels (*Camelus dromedarius*) of Iran. J Vet Res. 58:97-100.

4.**Rojo-Vázquez F.A., Meana A., Valcárcel F., Martínez-Valladares M.**(2012). Update on trematode infections in sheep. Vet Parasitol. 189:15-38.

5.O**laechea F., Lovera V., Larroza M., Raffo F., Cabrera R.**(2011). Resistance of *Fasciola hepatica* against triclabendazole in cattle in Patagonia (Argentina). Vet Parasitol. 178:364-366.

6.**Moll L., Gaasenbeek C.P.H., Vellema P., Borgsteede F.H.M.**(2000). Resistance of *Fasciola hepatica* against triclabendazole in cattle and sheep in The Netherlands. Vet Parasitol. 91:153-158.

7.**Boray J.C., Crowfoot P.D., Strong M.B., Allison J.R., Schellenbaum M., Von M.O., *et al.***(1983). Treatment of immature and mature *Fasciola hepatica* infections in sheep with triclabendazole. Vet Rec. 113:315-317.

8.**Fairweather I.**(2005). Triclabendazole: new skills to unravel an old (ish) enigma. J Helminthol. 79:227-234.

9.**Maes L., Vanparijs O., Lauwers H., Deckers W.**(1990). Comparative efficacy of closantel and triclabendazole against *Fasciola hepatica* in experimentally infected sheep. Vet Rec. 127:450-452.

10.**Rasheed S.A., Kadir M.A.**(2008). Prevalence of some parasitic helminths among slaughtered ruminants in Kirkuk slaughterhouse, Kirkuk, Iraq. Iraqi J Vet Sci. 22:81-85.

11.**Hassan H.A., Majid R.A., Rashid N.G., Nuradeen B.E., Abdulkarim Q.H., Hawramy T.A., *et al.***(2013). Eosinophilic granulomatous gastrointestinal and hepatic abscesses attributable to basidiobolomycosis and fasciolias: a simultaneous emergence in Iraqi Kurdistan. BMC Infect Dis. 13:91.

12.**Abed F.M.**(2012). A Pathological study of lesions in the liver of sheep in abattoir of Kirkuk province. Europ J Appl Sci. 4:140-145.

13.**Meerkhan A.A., Razak A.H., Younis B.M.** (2013). The differences between Direct examination and enzyme linked immunosorbent assay (ELISA) test, during the diagnosis of fasciolosis in Jaundiced slaughtered sheep in Duhok Abattoir, Kurdistan Region of Iraq. Int J Chem Env Biol Sci. 1:707-709.

14.**Kelley J.M., Elliott T.P., Beddoe T., Anderson G., Skuce P., Spithill T.W.** (2016). Current threat of triclabendazole resistance in *Fasciola hepatica*. Trends Parasitol. 32:458-469.

15.**Veenendaal G.H., De Waal M.J.**(1974). Uncoupling activity of the anthelmintic oxyclozanide in rodents. Br J Pharmacol. 50:435-437.