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. This study 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 studies have been conducted, and no evidence of resistance of F. hepatica against triclabendazole and closantel has been reported yet. A sheep farm in Sharazor district, Sulaymaniyah city, Kurdistan region was previously treated with triclabendazole but the therapy failed to clear the 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 Aghach village that is located in the northern part of Sharazor district, 43 km southeast of Sulaymaniyah 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 *Lymnaea atrun 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

\[ \frac{EPG \text{ day } 0 - EPG \text{ day } 14}{EPG \text{ day } 0} \times 10. \]

The 95% confidence interval (CI) was calculated as

\[ \left\{ \frac{EPG \text{ day } 0 - EPG \text{ day } 14}{EPG \text{ day } 0} \times 100 \right\} \pm 1.96 \sqrt{Y2}, \]

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).
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).

Figure 1 Administrative map of Sulaymaniyah, showing the location of Tut Aghach village.
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 in Iraq(12, 13). Anthelmintic therapy 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 Sharazor against 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 treat fasciolosis 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, no previous study about 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 are salicylanilide anthelmintics used 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 nitrozinil 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.

مقامة الدودة الكبدية *Fasciola hepatica* مقاومة ضد تريركلابينيدازول، كلوزانتيل ورافوكسانايدي بمزرعة في قضاء شهرزور في كردستان-العراق

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الخلاصة

من أهم الإصابات بالديدان الطفيلية في *Fasciola hepatica* لطالما كان مرض تفعن الكبد الذي يسببه الدودة الكبدية في العراق. وتستند السيطرة على هذا الطفيلي إلى استخدام مضادات الديدان مثل ترايكلابينيدازول وكلوزانتيل. توفرت مجموعة من الخراف في مزرعة بقرية توت أغاج في قضاء شهرزور في السليمانية بالعراق جراء مرض تفعن الكبد على الرغم من العلاج السابق باستخدام ترايكلابينيدازول. وقد أجريت هذه الدراسة لتبين تأثير ثلاثة أدوية متاحة تجاريا – ترايكلابينيدازول، كلوزانتيل ورافوكسانايدي – ضد عدوى تفعن الكبد الطبيعية في الأغنام. تم استخدام اختبار الحد من عدد البيض في البراز (FECRT) مع فاصل ثخن منخفض قدره 100 %، كما خفض كلوزانتيل ورافوكسانايدي من مزرعة غنم في *F. hepatica* المعالجة بنسبة 70.1 % و 70.2 %، على التوالي. تؤكد هذه الدراسة على مقاومة نموذجية *F. hepatica* لعلاج الدودة الكبدية في السليمانية، العراق ضد كلوزانتيل ورافوكسانايدي وترايكلابينيدازول. هناك حاجة إلى دراسة المزيد من المزارع في المناطق الموبوءة في العراق لتقييم مدى ظهور مقاومة ضد الأدوية للديدان الطفيلية.
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