Lack of reversion in triclabendazole-resistant *Fasciola hepatica*

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RESISTANCE to triclabendazole in field isolates of the liver fluke *Fasciola hepatica* in sheep was described for the first time by Overend and Bowen (1995) in Australia. In Europe, resistance of *F hepatica* in sheep to triclabendazole has been reported in Ireland (Lane 1998), Scotland (Mitchell and others 1998), Wales (Thomas and others 2000) and the Netherlands (Moll and others 2000). On the Dutch farm where resistance was found, the resistant flukes were present in sheep and cattle.

After the demonstration of triclabendazole resistance on the Dutch farm in the winter of 1998/99, resistance to the drug was also observed in the same season on three other farms in the vicinity of the first farm. In the autumn and winter of 1999/2000, triclabendazole resistance was found on four other farms, in 2000/01 on five farms and in 2001/02 on one farm. Studies on the suspected cases in 2003 and 2004 are ongoing. At the time of writing, the total number of Dutch farms where resistance to triclabendazole has been found and proven by a faecal egg count reduction test was 14. All the farms are situated in the same area, and the greatest distance between any two farms is less than 30 km. The farms are located in the province of North Holland, in an area that is below sea level, with ideal conditions for survival of a large population of the mud snail *Lymnaea truncatula* even in 'dry' summers. Therefore, farmers need to treat their sheep with fasciolicides several times per year. Following the demonstration of resistance to triclabendazole, farmers were advised to use a fasciolicide with a different mode of action. In the Netherlands, triclabendazole is the only fasciolicide registered for use in sheep, but in proven cases of resistance, closantel may be used under strict conditions and with the guidance of a veterinarian.

In order to investigate whether there was a reversion towards susceptibility to triclabendazole, in 2002 the authors returned to the farm where resistance had first been demonstrated in 1998/99 (Moll and others 2000). Since the demonstration of resistance on the farm, the farmer had treated his sheep each year with closantel; generally, three treatments per year were given, in the spring, autumn and winter. In autumn 2002, the farmer had sold all his sheep and bought new animals from a farm where liver fluke had never been found; these liver fluke-free sheep were grazed on his pastures from October 2002 onwards.

In December 2002, blood samples from five sheep were taken to check for the presence of antibodies by means of a *F hepatica*-specific serological test (Cornelissen and others 1992). All the sheep proved to be serologically positive, but coprologically negative by the Dorsman (1956) technique. This was not surprising, because the flukes were still in the prepatent period. In February 2003, the same five sheep were coprologically positive (range 2 to 50).

In March 2003, the efficacy of triclabendazole was tested in 10 sheep (Table 1); the triclabendazole treatment had no significant effect on the egg output after seven days. Therefore, a second treatment, with closantel, was given; this treatment proved to be effective. The efficacy of closantel against triclabendazole-resistant *F hepatica* has been demonstrated by Coles and others (2000) and Moll and others (2000).

TABLE 1: Fluke egg counts in sheep before and after treatment with triclabendazole and closantel in 2003

Sheep	Faecal egg count on March 5*	March 12	March 25†
1	15	15	10
2	30	15	0
3	380	380	0
4	730	430	0
5	740	1020	0
6	25	45	0
7	450	350	0
8	570	450	0
9	280	150	0
10	230	260	0
Mean	345	312	1

* Treatment with 10 mg/kg bodyweight triclabendazole
† One week after treatment with 10 mg/kg bodyweight closantel on March 19

This study showed that three years of usage of a fasciolicide with a different mode of action to that of triclabendazole did not result in reversion, that is, a return to susceptibility to triclabendazole. This result is comparable to that reported in the literature on the lack of reversion to susceptibility to benzimidazole in resistant nematodes (Borgsteede and Duyn 1989). However, care must be taken when comparing reversion in nematodes and trematodes. In nematodes, there may susceptible infective larvae in refugia. With trematodes, which undergo asexual multiplication in the intermediate host, selection for resistance and the elimination of genes for susceptibility may develop more rapidly. This emphasises the need to stop using a drug before a total failure of efficacy and the impossibility of a return to susceptibility due to a lack of 'susceptible' genes. In such a scenario it may be possible to reintroduce susceptible flukes from other areas, as has been shown for *Haemonchus contortus* (van Wyk and others 2001).

The time between clearance of the farm of the original sheep and restocking was far too short to have had any effect on infection by resistant fluke in the bought-in sheep. It would be worth investigating this strategy, while giving sufficient time for the encysted herbage larvae and those within snails to die off. However, wildlife, such as rabbits and hares, might harbour and ensure the continued existence of the resistant strain of *F hepatica*.

The present results, when combined with the recently observed increase in fasciolosis in sheep and cattle in the UK (Daniel and Mitchell 2002), indicate that there is an urgent need for research and consultation on the correct use of available fasciolicides and alternative control strategies, such as lowering the ground water level, good drainage, cleaning of ditches, pasture management, alternative land use and avoiding or fencing off pastures or parts of the pastures at risk. Thought should also be given to the identification of biological and other control measures for mud snails and pasture larvae.

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