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CHEMOTHERAPY IN OVINE MALIGNANT THEILERIOSIS *

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Treatment of diseases caused by *Theileria* species had been the aim of researchers since the end of the last century when the causative agents became known. Several chemicals and copounds had been tried (Barnett, 1968; Joyner & Brocklesby, 1973) mainly unsuccessfully but a few claims of limited success had also been made. The bulk of work had been carried out by research workers in South and East Africa, mainly Kenya, on East Coast fever (ECF). The first report of discriminatory effect of a drug tested in controlled trials against ECF came from Neitz (1950) when he published the results of work on 8–aminoquinoline compounds; pamaquin, pontaquin and primaquin against erythrocytic forms of *T. parva* and *T. mutans* and the 4–aminoquinolin chloroquin either alone or with pamaquin against the schizontal stages of *T. parva*. Later on Neitz (1953) described the suppressive effect of chlortetracycline when administered throughout the incubation of ECF. Still at a later date a similar effect was observed with oxytetracycline (Neitz 1957). Wilde (1967) in an intensive work screened 170 compounds against ECF in cattle and remarked that in spite of many earlier claims to success no

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authenticated cure was found. According to him, apart from tetracyclines which could give reproducible results, the folate antagonist pyrimethamine had discernible effect which could be improved when combined with sulphonamides.

There was a breakthrough in the chemotherapy of theileriosis when McHardy *et al* (1976) and Dolan & McHardy (1978) reported the curative effect of menoctone against clinincal cases of ECF. The exploitation of the *in vitro* culture of Theilerial schizonts for the screen of chemical compouns provided, as Wilde (1967) had hoped, a convenient means for test of new drugs at a lower cost. Menoctone that had been found *in vitro* to be effective against schizontal stages of both *T. parva* and *T. annulata* could not be further developed due to economical reservations and complexity to synthetise. Instead an analogue which did not possess the afore mentioned dismerits had to be searched and, fortunately, one was found and coded 993C for which, later, the name of Clexon (parvaquone Wellcome) was coined.

Schein and Voigt (1979) anounced the anti-Theilerial activity for the drug halofuginone hydrobromide (Stenerol Hoechst) which had been found by Manuel *et al* (1977) to be effective against leucocytozoonosis. Halofuginone that had been extensively used for prophylaxis of poultry coccidiosis (Yvore *et al,* 1974; Ryley & Wilson, 1975; Bedrnik *et al* 1979), is a synthetised halogen alkali of the active principle, Febrifugine, which is found in the roots and leaves of the plant *Dichroa febrifuga.* This herb had been used as a remedy for malaria in China for more than two thousand years. According to these authors the drug at the rate of 1.2 mg./Kg. b. w. was schizonticidal but did not affect the erythrocytic forms.

Apparently the prospective of coming by a real therapeutic agent that could be used in clinical cases of theileriosis has never been so promising as it is now and as Brown & Masiga (1981) put it, there are several compounds in the pipeline. However, despite extensive work that

is done in the field of treatment of cattle theileriosis, virtually nothing could be revealed, from the perusal of literature, in relation to sheep malignant theileriosis. This disease which is limited to certain parts of the world is characterised by hyperthermia, anaemia, jaundice, emaciation and a high rate of mortality. The causative agent is the protozoan parasite *T. hirci.* It is a tick-borne disease and the vector is *Hyalomma a. anatolicum* of Ixodid ticks (Hooshmand - Rad & Hawa, 1973).

Given that the therapeutic effects of the recently developed compounds did not greatly vary against either *T. parva* or *T. annulata*, it was considered reasonable for them to be tried against *T. hirci* infection. The results of trials on parvaquone and halofuginone are presented in this paper. As therapeutic values had been locally claimed for drugs oxytetracycline and quinuronium sulphate, these two drugs were tried in field cases of sheep malignant theileriosis and the results are also included.

MATERIALS AND METHODS

Sheep: Seven to ten monts old local fat-tailed breeds were used. Blood smears of the animals in experiments 1 and 2 had been meticulously searched for blood protozoan parasites prior to the start of the experiments.

Theileria hirci strains: This which is designated THSI had been isolated from a field case of theileriosis and had been maintained in carrier sheep.

Ticks: Hyalomma a. anatolicum were bred at the laboratory according to Hooshmand-Rad & Hawa (1973). Infestation of the sheep with infected ticks was carried out according to Bailey (1961). All ticks had been infected at the nymphal stage.

Body temperature: Rectal temperatures of the experimental sheep were recorded once daily around midday. Temperatures of 40°C. or above were taken as pyrexia.

Lymph node biopsies and blood smears: Biopsies were carried out on

animals showing pyrexia. This was continued daily till animals death or complete disappearance of the parasites from lymph nodes. Blood smears were prepared on day 0 and then on every other day. All smears were fixed with methanol and stained with Giemsa stain.

EXPERIMENTS AND RESULTS

Experiment 1: Eight sheep divided into two 4-sheep groups were infested with 20 ticks (10 male & 10 female). Animals in group 1 received Stenerol (Hoechst) at the rate of 1.4 mg. Kg. b.w. of the active ingredient halofuginone hydrobromide on day 3 of pyrexia with patency of schizonts in the lymph nodes. Temperatures dropped and schizonts showed degeneration within 24 hours but a second dosing became necessary in one case after 24 hours and in the other 3 after 72 hours. All animals got rid of the parasites after the second dosings. Two of the sheep in the control group died of theileriosis while the other - recovered only after a relatively protracted period of parasitosis. The results are summarrized in the Table.

Experiment 2: Eight sheep divided into two 4-sheep groups, were infested with 20 ticks infected with *T. hirci.* Animals in the first group received parvaquone at the rate of 20 mg./kg. b.w. in the muscles of the neck, on the third day of pyrexia and patency of schizonts in the prescapular lymph nodes. Pyrexia subsided and barasites disappeared from the lymph nodes in 48 hours after parentral administration of the drug. Three of 4 sheep in the control group died of theileriosia and the fourth one survived. Results are summarized in the Table.

Experiment 3: In an outbreak of theileriosis lambs afflicted with the disease were divided into 3 groups, each of 8 sheep. Group 1 received daily doses of 10mg. /Kg b.w. of oxytetracycline intramuscularly. Group 2 received a single dose of 0.5 mg./Kg.b.w. quinuronium sulphate

subcutaneouly. Group 3 received daily doses of 10ml. Physiological saline. No conceivable effect could be noticed and all the animals eventually died of the disease. The results are summarized in the Table.

DISCUSSION

The effectiveness of the drugs was judged on the basis of death and recovery rates as well as parametres such as the number of days in pyrexia and the patency of schizonts. It is not certain that the number of animals used in the experiments was large enough to meet the requirements for statistical analysis. It had been suggested (Brocklesby *et al* 1961) that in using infective material which produced a morbidity of 87% and a mortality of 95.5% a minimum of 7 animals per group should be used if death or recovery were to be statistically significant criteria of effect. The strain used in the present experiments had not been fully characterised. The use of stabilates as that described for *T. parva* (Cunningham, 1970; 1973) which makes the prediction of the resulting infections more accurate, though exploited with success for *T. hirci* at our laboratory has not been made routine so far. Much work is still needed to clearly define the degree of susceptibility of the local breeds and the existence of variations if there is any at all

Although parvaquone and halofuginone were used at the dosal rates recommended for cattle theileriosis significant degrees of beneficial results were gained. Future research should be guided toward the determination of the therapeutic as well as toxic dosages of these drugs in sheep. The dismerit that had been observed with halofuginone of causing diarroea (Schein & Voigt, 1981) was not noticed in the present experiments, perhaps due to smallness of the animal poulation treated. Moreover, the future research should include the antibioties in the ionophores group for which antitheilerial activities have been acclaimed.

The effect of drugs parvaquone halofuginone, oxytetracycline and quinuronium sulphate against T. hirci

Experiment No.	Group No.	Drug	Treatment	Mean duration of fever (days)	Survival proportion
1	1	Halofuginone	1.4 mg. /Kg* b.w.(orally) on Day TS3	4	4/4
	2	Nil	Nil	10 (8–12)	2/4
2	1	Parvaquone	20mg. /Kg. i/m on Day TS3	4.5 (4–5)	4/4
	2	Nil	Nil	11 (8–14)	1/4
3	1	Oxytetracycline	10mg. /Kg. i/m daily	8.1 (6–10)	0/8
	2	Quinuronium Sulphate	0.5mg /Kg s/c once	8.8 (8–10)	0/8
	3	Placebo	10ml. saline daily	8.5 (8–12)	0/8

* One animal received a second dose after 48 hours and the other 3 after 72 hours.

TS3: The day of pyrexia and the patency of schizonts.

It is hoped that sheep, being a smaller and therefore cheaper animal than cattle, would make a model animal for *in vivo* tests on antitheilerial drugs so far known, in the making or to be developed in the future.

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