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Elimination of *Eperythrozoon coccoides* infection from mouse colonies

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During the past 15 years the importance of *Eperythrozoon coccoides* Schilling, 1928, in certain infections of mice has been amply demonstrated. Infection with *E. coccoides* does not itself produce overt signs of ill-health, but it causes splenic hyperplasia and alters the responses of mice to other agents, sometimes with fatal results. Experimentally *E. coccoides* enhances the pathogenicity of mouse hepatitis virus so that fatal hepatitis ensues (Niven, Gledhill, Dick & Andrewes, 1952; Gledhill & Dick, 1955). *E. coccoides* also increases the pathogenicity of lymphocytic choriomeningitis virus inoculated peripherally, converting a mild immunizing infection into a fatal disease (Seamer, Gledhill, Barlow & Hotchin, 1961). Similarly, the parasite can increase the susceptibility of mice to the lethal action of Gram-negative bacteria and their endotoxins; during the phase when eperythrozoa are abundant in the blood, mice are about 100 times as susceptible to bacterial endotoxin as normal mice (Gledhill & Niven, 1957). Infection with *E. coccoides* increases the lactose dehydrogenase activity of the blood plasma in a way similar to the Riley agent (Arison, Cassaro & Shouk, 1963). The infection has also been shown to increase the phagocytic activity of mice, as judged by the uptake of intravenously inoculated carbon particles (D. L. J. Bilbey, A. W. Gledhill & J. S. F. Niven, unpublished data).

Since *E. coccoides* can readily be transmitted from infected mice by inoculation of blood, or blood-containing tissues, this potentially serious source of error should be eliminated from mice used for experimental purposes. Like other eperythrozoa, *E. coccoides* is believed to be transmitted under natural conditions by biting insects and particularly by lice (Eliot, 1936). In infected breeding colonies, experience has shown that the majority of mice become infected within the first months of life, and after a week or so during which parasitaemia is readily detected, infection may persist for 6 months or longer. The purpose of this communication is to show that *E. coccoides* infection can be eliminated from a mouse colony by regular insecticidal treatments designed to reduce infestation with the mouse louse, *Polyplax serrata*, to a very low level, if not to eradicate it completely.

MATERIALS AND METHODS

Mice. The breeding colony of Parkes (P-strain) mice has been maintained as a closed colony at the National Institute for Medical Research, Hampstead and Mill Hill, since 1922 and is known to have been infected with *E. coccoides* for more than 10 years. Lice were occasionally observed on P-strain mice before the work

to be described. In testing for the presence of *E. coccoides* in P-strain mice, mice of the TO-strain were used. This strain has been bred as a closed colony at the Institute since 1953 and was found not to be infected with *E. coccoides*.

Insecticidal treatment

A powder containing pyrethrum and piperonyl butoxide ('Pybuthrin', Cooper, McDougall and Robertson, Ltd.) was blown into each of the mouse boxes containing P-strain mice twice weekly for 6 weeks and thereafter once every two weeks.

Estimation of the proportion of mice infected with Eperythrozoon coccoides

Infection with *E. coccoides* is readily detected by examining blood smears taken daily for some 10 days after splenectomy of individual mice. However, in order to facilitate the examination of a representative proportion of the colony, an indirect method based on the enhancing effect of *E. coccoides* upon mouse hepatitis virus was used, the enhancement being recognized by fatal hepatitis or by degrees of liver damage greater than in normal mice inoculated with virus alone. Ten groups of ten P-strain mice aged 21 days were taken at random from the colony. (At this age it had been our general experience that about 25 % of mice are infected, whereas at 10–12 weeks of age 50–100 % are infected.) The mice were killed and the spleens from each group were removed and emulsified in 10 ml. of chilled serum saline broth (10 % horse serum, 45 % beef infusion broth, 45 % normal saline). After light centrifugation, 0·2 ml. inocula of the supernatants were injected intraperitoneally into groups of five TO-strain mice aged 18–21 days. On the following day the mice received an inoculation of about 100 ID50 mouse hepatitis virus (MHV 1); a control group of mice which received virus only was included in each experiment. All mice that died were autopsied and 6 days after the virus inoculation the surviving mice and the controls were killed and autopsied. Macroscopic liver lesions were scored according to a scheme described by Gledhill (1961) as follows:

Appearance of liver	Score
No obvious focal lesions	0
A few focal lesions	1
Many focal lesions	2
Coalescence of focal lesions to give generalized liver abnormality	3
Liver bright yellow with haemorrhagic areas	4
Mouse dead with liver as in last group	5

Enhancement of MHV 1 as evidenced by severe macroscopic liver damage (score 3 or greater) in most mice of a group was taken to indicate the presence of *E. coccoides* in at least one of the P-strain mice that provided the inoculum of spleen suspension; and in the absence of such damage it was considered that none of the P-strain mice was infected with *E. coccoides*. With these assumptions it was possible to give an estimate of the proportion of mice infected with *E. coccoides* among the 100 examined. This procedure was carried out when treatment began and after 6, 15 and 30 weeks.

RESULTS

The results of the first test, made when treatment began, are shown in the upper half of Table 1. On the basis that inoculation of MHV 1 into normal mice caused liver lesions that scored two or less, it is apparent that the pathogenicity of the virus was enhanced in nine groups of mice, and only one group was considered to be free of infection with *E. coccoides*. Calculations based on the binomial distribution show that an infection rate of 21 % among the 100 P mice examined would

Table 1. *The incidence of* Eperythrozoon coccoides *infection in weanling P-mice* (*a*) *before and* (*b*) *after* 6 *weeks of insecticidial treatment*

Groups of five weanling mice	Liver score at autopsy 1	2	3	4	5	Total	*E. coccoides* infected
			(*a*) Before treatment				
1	1	1	1	0	0	3	0
2	5	4	3	3	0	15	+
3	4	4	4	3	2	17	+
4	4	3	3	3	0	13	+
5	4	4	4	3	3	18	+
6	5	5	5	5	3	23	+
7	5	4	4	4	0	17	+
8	5	5	3	3	1	17	+
9	4	4	4	3	0	15	+
10	4	4	4	4	0	16	+
			(*b*) After 6 weeks treatment				
1	5	5	4	4	0	18	+
2	1	1	0	0	0	2	0
3	5	5	4	4	3	21	+
4	2	1	0	0	0	3	0
5	2	2	1	0	0	5	0
6	2	2	1	0	0	5	0
7	1	0	0	0	0	1	0
8	5	4	4	4	2	19	+
9	2	0	0	0	0	2	0
10	2	1	0	0	0	3	0

(1) Each group of five weanling TO mice inoculated i.p. with 0·2 ml. 10 % spleen suspension from ten weanling P-mice and 1 day later 0·2 ml. i.p. liver suspension containing about 100 ID 50 MHV 1.

(2) Mice which died autopsied and scored for hepatitis. Survivors at 6 days after virus inoculation killed, autopsied and likewise scored.

(3) The system of scoring liver damage is stated under methods.

be most likely to give this result and would in fact give it in 39 % of tests. If, however, the actual infection rate was less than 7 % or greater than 50 % the result obtained would be expected in less than 1 test in 100. These inferences accord also with the observation that twenty-two spleens from the original 100 P mice were considered to be enlarged, while the remainder were considered to be of normal size or small.

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The second test was carried out at the end of the 6-week period of bi-weekly treatment, and the results appear in the lower half of the table. A clear distinction was again apparent between three groups in which the pathogenicity of MHV 1 was enhanced and seven groups in which it was normal. Similar calculations show that an infection rate of 3·5 % would be most likely to give the result obtained, and would in fact give it in 27 % of tests. If the actual infection rate was less than 0·5 % or greater than 11 % this result would be expected in less than 1 in 100 tests.

The third and fourth tests were carried out 9 and 24 weeks after the second test, during which time the treatment was applied once fortnightly. In both these tests the pathogenicity of MHV 1 was within normal limits in all groups, and it was inferred that none of the donors were infected with *E. coccoides*.

The results suggested that treatment against lice had prevented the spread of *E. coccoides* to mice born after the treatment was started. In order to obtain further evidence that infection with *E. coccoides* was no longer present in the colony, twenty-one female P-strain mice aged about 12 weeks were selected at random after treatment had been given for 1 year. These mice were splenectomized, and blood smears were taken from each on the 3rd, 6th, 7th, 8th, 9th, 10th, 13th and 15th days after operation. No eperythrozoa were seen in the 168 blood films examined.

DISCUSSION

We assume from these results that after 6 weeks of intensive and 9 weeks of fortnightly insecticidal treatment, the spread of *E. coccoides* to mice born within the infected colony had been stopped. No doubt some older mice were still infected with eperythrozoa at this time, for Derrick, Pope, Chong, Carley & Lee (1954) found that infection persisted for 4 months in some mice, and we have experimental evidence to support this finding. However, although some mice are retained in the colony for breeding purposes for as long as 9 months, there appeared to be no spread from the long-term carriers, for the splenectomies performed 1 year after treatment began failed to reveal infection with *E. coccoides* in mice of an age group in which the incidence would previously have been 50–100 %.

We have evidence that large doses of *E. coccoides* will infect a proportion of mice when given *per os* (J. Seamer, unpublished observations), and transmission by this method might possibly become significant in colonies in which deaths were frequent and cadavers often eaten, but this was not the case in the P colony. As to the ectoparasites concerned, it is known that *E. coccoides* can be transmitted by lice (Eliot, 1936; G. W. A. Dick, personal communication), and lice had been observed on the P mice. Fleas were not observed, but in any event the insecticide should have been as effective against fleas as against lice. Mites which have been observed on the mice were unlikely to be responsible for the maintenance of infection since the insecticide is not effective against them. Although *E. coccoides* seems to have been eliminated from our colony, we do not assume that lice have been entirely eliminated; it is possible that reduction of the louse infestation below a critical level would effectively curtail the transmission of the eperythrozoa.

The use of MHV 1 to indicate the presence of *E. coccoides* enabled the proportion

of infected mice at the various stages of treatment to be estimated more quickly than by the direct method of splenectomy and examination of blood smears. However, certain murine leukaemia agents also enhance the pathogenicity of MHV 1 (Gledhill, 1961) and inferences based upon results obtained with the virus require at some point to be confirmed by observations of blood smears.

The necessity of providing mice free of *E. coccoides* for experimentation has been emphasized and, on the basis of our experience, it would not appear difficult to provide such mice. Measures to control lice by insecticidal treatment should be carried out as a routine in all conventional colonies. Suspected infection with *E. coccoides* in a colony should be proved by the daily examination of blood smears for 10 days from a dozen or so splenectomized mice. If infection is demonstrated the test should be repeated after about 6 months with another group of mice, to assess the effect of prophylactic measures. For these tests mice about 3 or 4 months old are most suitable since an infection rate representative of the colony will not have developed in very young mice and will be more difficult to demonstrate with increasing age. In this connexion the fact should be stressed that *E. coccoides* is readily transmissible to intact mice and that parasitaemia is easily demonstrable; indeed, we have utilized this characteristic of *E. coccoides* as the basis of much experimental work over many years (Niven *et al.* 1952; Seamer, 1959).

SUMMARY

1. Long-established infection of a mouse colony with *Eperthyrozoon coccoides* was eliminated by regular insecticidal treatments designed to reduce infestation with lice and fleas.

2. Undesirable consequences of *E. coccoides* infection upon mice used as experimental animals are noted and an easy routine for exclusion of this parasite from mouse colonies is suggested.

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