Onderstepoort Journal of Veterinary Research,
Volume 26, Number 2, November, 1953.
The Government Printer, Pretoria.

THE PARASITICIDAL EFFECT OF AUREOMYCIN (LEDERLE) ON *BABESIA EQUI* (LAVERAN 1899) IN SPLENECTOMISED DONKEYS.

B. C. JANSEN, Onderstepoort Laboratory.

Veterinarians in countries where tick-borne diseases are prevalent are well-acquainted with the difficulties encountered in the treatment of equine Babesiosis (= Nuttaliosis). Affected animals do not respond satisfactorily to the present methods of treatment in all cases.

In the systematic search for more effective drugs with a specific action against *B. equi*, Aureomycin, with its wide therapeutic spectrum, was tested for its babecidal action. The drug was kindly supplied by Lederle Laboratories Division, American Cyanamid Company, to whom it is desired to express our appreciation of their cooperation.

Materials and Methods.

Due to the wide distribution of the natural vector of *B. equi* in South Africa, viz. *Rhipicephalus evertsi* (Neumann 1897) great difficulty was experienced in obtaining susceptible horses for these experiments. Eventually it became necessary to breed susceptible experimental animals under tick-free conditions. Horses were considered rather too large to be kept under tick-free conditions for lengthy periods so donkeys were substituted.

Donkey mares in advanced pregnancy were thoroughly cleaned and sprayed with a solution of benzenehexachloride (200 parts of the gamma isomer per million) before being placed in loose boxes to foal. The dams and foals were cleaned and sprayed regularly, and were maintained throughout the experiments under tick-free conditions. The precautions adopted were effective in preventing accidental tick infestation.

As in the case of other protozoal diseases, young animals do not suffer from the pathogenic effects of *B. equi* to the same extent as adult animals. Keeping these donkey foals to maturity would prolong the experiment unnecessarily. The solution seemed to lie in splenectomy, to increase the susceptibility of the foals to the desired degree. The operation was carried out according to the technique described by Quinlan, de Kock and Marais (1935), and although the equine is notorious for its susceptibility to peritonitis, none were lost from this cause.

Experimental.

A. *To Determine the Pathogenicity of* B. equi *for young Donkeys.*

A three months old donkey foal was given intravenously 10 c.c. of blood from a donkey which was known to harbour *B. equi*.

Received for publication on 7th April, 1953.—Editor.

On the tenth day a few parasites were seen in blood smears from the foal but none could be found the following day. The temperature never rose above 101·4° F. and at no time were there any clinical symptoms of ill health.

Conclusion.

This experiment on a single animal supports previous observations that the resistance of the donkey foal to *B. equi* infection is sufficiently high to exclude the possibility of its use as an experimental animal for chemotherapeutic studies.

B. *To Determine the Pathogenicity of* B. equi *for splenectomised young Donkeys.*

I.—A donkey foal, D.O.B. 1876, was splenectomised on 26th April, 1950. Blood smears were negative for the following 26 days when it received intravenously 10 c.c. blood from a donkey known to be premune to *B. equi*. Five days later blood smears showed the presence of parasites and several Maltese Cross forms characteristic of *B. equi* during active multiplication, were seen for the first time. Two days later the blood was swarming with parasites. The following morning the foal died i.e. on the third day after parasites were detected.

II.—A second donkey foal, D.O.B. 2178, showed no parasites in blood smears for a month after splenectomy, when it received intravenously 20 c.c. of blood from a horse that had recovered from an attack of biliary fever. After an incubation period of nine days a few parasites were found in blood smears. The foal died four days later with almost every erythrocyte invaded by parasites.

Conclusion.

It is concluded that the susceptibility of the splenectomised babesia-free donkey foal is such that any effect of a drug upon the parasite should be detected easily and prevention of a fatal termination of infection should be significant.

C. *To Determine the effect of Aureomycin on* B. equi *in splenectomised Donkey Foals.*

I.—On 18th August, 1950, a donkey foal, D.O.B. 2019, weighing 133 lb. which had been splenectomised three weeks previously, received infective blood intravenously. Blood smears were examined daily but no parasites were found until the seventh day. Immediately they were detected 1 gram of aureomycin was injected intravenously (0·5 mgm. per lb.) and the dose was repeated the following day. The day after the second treatment it was apparent from the examination of blood smears that although the parasites had increased in numbers many showed degenerative changes as evidenced by a pyknotic appearance of the nuclei and loss of a definite outline to the cytoplasm. Twenty four hours later only degenerative forms could be found. It appeared as if the course of the infection had been arrested but two days later actively multiplying parasites showing typical Maltese Cross divisional forms reappeared. Treatment with the same dose of aureomycin was repeated and the same cycle of events followed, i.e. an initial degeneration of the parasites followed later by multiplication and an increase in numbers. However, it was apparent that the progress of infection was being interrupted and treatment was delayed for a period of 10 days. On that day and again 2 days later 1 gram aureomycin was administered intravenously but the day after the last treatment, the 21st day after infection and the 14th day after parasites were detected for the first time in smears, the foal died.

Conclusion.

The effect of the aureomycin in arresting the progressive multiplication of the parasites was apparent from the daily examination of the blood smears. Moreover the course of the disease was prolonged considerably because death was delayed until the 14th day after the parasites first appeared whereas the two untreated controls were both dead by the fifth day. The total of 6 grams of aureomycin divided into 6 equal doses at irregular intervals was inadequate to suppress the parasite completely. It was important, therefore, to determine the effect of larger doses of the drug.

II.—A donkey foal, D.O.B. 1784, weighing 212 lb., was infected two weeks after splenectomy. On the third day of reaction, when about 30 per cent of the erythrocytes were parasitised and Maltese Crosses were frequent, the animal received an intravenous injection of 2·5 grams of aureomycin in 1% solution i.e. 11·8 mgm. per pound body weight. The injection was given slowly and only caused slight shivering on the part of the donkey. On the following two days the dose was repeated. On the third day the parasites were considerably decreased in numbers and showed degeneration. At varying intervals the treatment was repeated on twelve occasions (c.f. appendix I) by which time no parasites could be found in the blood smears. For a time it was thought that complete sterilisation had been effected but a relapse occurred after an interval of 14 days. After four daily injections of 3·0 grams of aureomycin (i.e. 14·2 mgm. per pound body weight) in 1% solution, the blood smears again became negative and no parasites were seen for two months, when a second relapse occurred. By this time supplies of the drug were exhausted and no further treatment could be applied. The foal died two days later, one hundred and thirty-nine days after parasites were first seen in blood smears.

Conclusion.

Aureomycin administered daily at the rate of 11·8 and 14·2 mgm. per lb. was effective in controlling the development and multiplication of *B. equi* in a donkey foal splenectomised two weeks prior to infection. Even at this level of dosage, however, sterilisation was not effected. After the first course of treatment which controlled multiplication of the parasite a relapse occurred. This in turn was successfully controlled at the higher dosage but a second relapse which was allowed to run its course without treatment proved fatal.

III.—Two months after splenectomy, during which time no parasites appeared in the blood, a donkey foal, D.O.B. 2151, weighing 173 lb. was given infective blood intravenously. After an incubation period of ten days parasites appeared in the blood smears on 13th May 1951. Treatment at the rate of 2·6 grams of aureomycin intravenously (15·6 mgm. per pound) was commenced and repeated on each of 6 successive days (c.f. appendix II). On the second day of treatment the temperature had risen to 105·2° F. but within 4 days returned to normal by lysis. During this period the donkey showed marked listlessness, inappetence and well defined icterus. Daily smear examination showed a marked increase in the number of parasites for the first three days in spite of treatment, after which they decreased in numbers progressively until they had disappeared by the ninth day. The general condition of the animal then improved rapidly and no parasites were detected for a month when a relapse occurred. Treatment was again applied at the same dosage (2·6 grams) repeated daily for four consecutive days. It was apparent that progress of infection was retarded. The foal remained in apparent good health, but the parasites disappeared very slowly. It was not until the 4th

July that the first negative blood smear was recorded. No parasites were seen for a full three weeks when a single parasitised cell was detected. From that time onwards odd *B. equi* have been found almost daily though never in large numbers and actively dividing forms were never encountered. A state of premunition had developed and the animal remained in continued good health.

Conclusion.

In this experiment the administration of aureomycin at the rate of 15·6 mgm. per lb. for six consecutive days effectively controlled the development of *B. equi*. Sterilisation was not effected but a relapse did not occur for a month when four daily administrations of the drug was again effective. No further relapse occurred.

Comment.

The role of the spleen in enabling an animal to develop a state of premunition or labile infection is well known and is clearly indicated by the flare up which follows splenectomy. It is also known that after splenectomy this function of the spleen is gradually assumed by other organs e.g. by the liver in which foci of splenic tissue develop. In the third experiment it will be noted that infection of the splenectomised foal was delayed until two months after splenectomy whereas in the other cases detailed the interval was not longer than 3 weeks. In addition the relapse was delayed for a month. The end result was that the drug was able to control the parasite which in turn had developed no apparent drug resistance. In 3 months and 3 weeks after splenectomy the normal defence mechanism of the body could operate to bring about a state of premunition.

The experimental work to this stage had indicated clearly the specific parasiticidial action of aureomycin. It was considered desirable to attempt to compare the relative effectiveness of aureomycin and Gonacrin (May & Baker) which is the drug of choice today for the treatment of babesiosis of horses.

A. *To Determine the effect of Gonacrin on* B. equi *in splenectomised Donkey Foals.*

I.—A donkey foal, D.O.B. 2017, weighing 200 lb. was splenectomised; after an interval of 15 days it received an intravenous injection of infective blood. On the 6th day rare *B. equi* were detected in the blood and Gonacrin was injected intravenously the dose being 6 c.c. This injection was repeated on the following two days but the parasites increased in numbers and degenerating forms were rare. The number of parasites decreased from the fourth day so that by the sixth day they would be described as rare. On the 7th day the infection flared up and in spite of two injections of 6 c.c. Gonacrin on successive days the donkey died. At the time of death the percentage of degenerative forms of the parasite was small.

II.—As this failure might be attributed to insufficient dosage of Gonacrin a second donkey foal, D.O.B. 2016, weighing 160 lb. was splenectomised for treatment with larger doses. On the second day of reaction it received 10 c.c. Gonacrin intravenously repeated on each of three successive days. The foal died on the day the last injection was given and the parasites did not decrease in numbers until the day of death when degeneration forms were detected for the first time in small numbers.

Conclusion.

Even four daily injections of 10 c.c. of Gonacrin was not effective for the control of the development and multiplication of *B. equi* in a splenectomised donkey weighing no more than 160 lb. The total volume of drug administered was considerably in excess of that which would be given to a foal of that size in practice where the therapeutic value of Gonacrin had been well established. The impression was gained that multiplication of the parasites was not the sole cause of the death of the foal but that toxicity of the drug was at least in part responsible. To throw some light on this point two further experiments were made.

B. *To Determine the toxicity of Gonacrin when administered repeatedly in large doses.*

I.—A non-splenectomised clinically healthy donkey foal of similar age and weight viz. 163 lb. was given 10 c.c. of Gonacrin intravenously on four successive days. It died on the ninth day and on post mortem examination showed marked degenerative changes of the liver.

II.—A second larger donkey weighing 372 lb. was given 20 c.c. of Gonacrin on each of six successive days i.e. the same dose per lb. body weight continued for two extra days. The foal became progressively weak and died on the day the sixth injection was given.

Conclusion.

Gonacrin administered by intravenous injection in daily divided doses of an amount insufficient to control *Babesia equi* in recently splenectomised donkey foals is toxic.

Discussion.

As far as is known splenectomy of the fully susceptible immature animal has been used for the first time in this series of experiments to produce a subject of value for the determination of the true therapeutic index of a drug against a pathogenic protozoon.

The therapeutic index of a drug is defined as the ratio of the minimum effective concentration or dose to the toxic concentration. It is determined usually by trial and error on a large number of individuals of assumed equal susceptibility in an extensive series of experiments. Usually the synergistic role of the response of the individual to infection such as rapid development of immunity or cellular resistance cannot be estimated.

The donkey foal was shown to be resistant to infection with *B. equi* but when infected as soon as reasonably possible after recovery from the surgical shock of splenectomy this resistance was removed with the result that the termination was invariably death. Consequently a technique was available for determining the *in vivo* parasiticidal value of a drug as distinct from the *in vitro* value which may be vastly different and of little practical significance. Any values determined by this technique are of importance but may indicate a specific effect considerably lower than those which would obtain in the intact susceptible animal.

In the first experiment of the series administration of aureomycin at the rate of 0·5 mg. per lb. undoubtedly had a clearly defined effect upon the development of the parasite. Progress of infection was assisted but the rapidity with which a relapse followed appeared to indicate that the dose was inadequate.

In the second experiment the dose was increased considerably—up to 14·2 mgm. per lb. Multiplication of the parasite was controlled but sterilisation was not effected. A relapse was delayed for a period in excess of 2 months. In the absence of further treatment this relapse proved fatal. It is reasonable to conclude that in that period the defence mechanism of the splenectomised animal could not be organised to assist the specific action of the drug and to establish a state of premunition. It must be remembered that in both these experiments infection was initiated as soon after splenectomy as possible.

In the third experiment infection was delayed until two months after splenectomy. The dose of aureomycin was increased only slightly to 15·6 mgm. per lb. Sterilisation was not affected but the incidence of a relapse was considerably delayed and eventually a true state of premunition developed.

From this experimental work it appears to be safe to conclude that the specific babecidal action of aureomycin has been demonstrated, although it was not possible to estimate the chemotherapeutic index of the drug. It is equally apparent however, that this index must be considerably wider than that of Gonacrin. It is a recommendation, therefore, that should a suitable opportunity occur aureomycin merits an extensive trial in general practice for the treatment of biliary fever in equines. With limited opportunities this work will be continued at Onderstepoort the dosage being at the rate of 2·5 mgm. per lb.

Acknowledgment.

My thanks are due to Mr. F. Boughton for his invaluable help in the performance of the operations and in the injection of the animals.

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APPENDIX I.

Donkey 1784. (Weight 212 lb.)

20. 8.50 Splenectomised.
11. 9.50 Injected with 10 c.c. infective blood.
18. 9.50 *B. equi* +.
21. 9.50 *B. equi* 3+, M.C., 2·5 gm. Aureomycin i.v.
22. 9.50 *B. equi* 4+, M.C. 2+, 2·5 gm. Aureomycin i.v.
23. 9.50 *B. equi* 4+, M.C. D. forms, 2·5 gm. Aureomycin.
24. 9.50 *B. equi* 3+, D.
25. 9.50 *B. equi* +, D.
27. 9.50 Blood negative, 2·5 gm. Aureomycin.
28. 9.50 Blood negative, 2·5 gm. Aureomycin.
25.10.50 Blood negative.
26.10.50 *B. equi* 2+, M.C.
27.10.50 *B. equi* 2+, M.C.

28.10.50 *B. equi* 3+, M.C., 2·5 gm. Aureomycin.
29.10.50 *B. equi* 3+, M.C., 2·5 gm. Aureomycin.
30.10.50 *B. equi* 3+, N. and D. forms, 2·5 gm. Aureomycin.
31.10.50 *B. equi* 4+, M.C. 2+, N. and D. forms, 2·5 gm. Aureomycin.
1.11.50 *B. equi* 4+, M.C. 2+, 2·5 gm. Aureomycin.
2.11.50 *B. equi* 5+, N. and D. forms, 2·5 gm. Aureomycin.
3.11.50 *B. equi* 5+, N. and D. forms, M.C. 2+, 2·5 gm. Aureomycin.
4.11.50 *B. equi* 3+, D. forms, M.C.+, 2·5 gm. Aureomycin.
5.11.50 2·5 gm. Aureomycin.
6.11.50 Blood negative, 2·5 gm. Aureomycin.
19.11.50 Negative.
20.11.50 *B. equi* 2+.
22.11.50 *B. equi* 4+, M.C., 3·0 gm. Aureomycin.
23.11.50 *B. equi* 7+, D. forms, 3·0 gm. Aureomycin.
24.11.50 *B. equi* 6+, M.C. 3+, 3·0 gm. Aureomycin.
25.11.50 3·0 gm. Aureomycin.
26.11.50 *B. equi* +.
27.11.50 Negative.
2. 2.51 *B. equi* 4+ M.C.
4. 2.51 Died.

NOTE:

+, 2+, 3+, etc. indicate degree of infection as seen in blood smears,

M.C. = Maltese Cross forms of division;

N. = Normal; and

D. = Degenerated.

APPENDIX II.

Donkey 2151. (Weight 173 lb.)

7.3.51 Splenectomised.
4.5.51 10 c.c. blood from 2123 i.v.
13.5.51 *B. equi* 2+.
14.5.51 *B. equi* 3+, M.C., 2·6 gm. Aureomycin.
15.5.51 *B. equi* 5+, M.C., (all D.), 2·6 gm. Aureomycin.
16.5.51 *B. equi* 4+, M.C., 2·6 gm. Aureomycin.
17.5.51 B. equi 3+, M.C., 2·6 gm. Aureomycin.
18.5.51 B. equi 3+, M.C., 2·6 gm. Aureomycin.
19.5.51 *B. equi* 3+, 2·6 gm. Aureomycin.
20.5.51 *B. equi* +.
21.5.51 Single D. forms of *B. equi*.
23.6.51 *B. equi* 2+.
25.6.51 *B. equi* 3+, M.C., 2·6 gm. Aureomycin.
26.6.51 *B. equi* 3+, D. forms.
27.6.51 *B. equi* 4+, 2·6 gm. Aureomycin.
28.6.51 *B. equi* 3+, 2·6 gm. Aureomycin.
29.6.51 *B. equi* 3+, 2·6 gm. Aureomycin.

30.6.51	Few degenerate specimens of *B. equi*.
2.7.51	Single degenerate forms of *B. equi*.
4.7.51	Negative.
24.7.51	*B. equi* +.
27.7.51	*B. equi* +.
31.7.51	*B. equi* +.
4.8.51	*B. equi* 2+.
8.8.51	*B. equi* 3+.
20.8.51	*B. equi* +.
1.9.51	*B. equi* 2+.
3.9.51	*B. equi* 3+, M.C.
12.9.51	*B. equi* +.
14.9.51	*B. equi* +.