

AUREOMYCIN IN THE TREATMENT OF HEARTWATER.

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Until Neitz (1939) reported that uleron, suitably administered, modified the course of heartwater in sheep no effective remedy for the treatment of this disease was known. Since the publication of that report a vast amount of additional work has been carried out with a wide variety of sulphonamides e.g. sulphanylamine, sulphapyridine, solupyrindine, sulphathiazole, sulphadiazine, sulphamerazine, sulphaguanadine, and with combinations of some of these drugs. It is not intended to publish the results in detail at this stage but it is safe to say that the therapeutic value of a sulphonamide is dependent upon the maintenance of an adequate blood concentration for the requisite period of time. The effective blood concentration is so low and the time so short in comparison with the requirements for the successful treatment of bacterial infections that the action on *Rickettsia ruminantium* may be regarded as almost specific in the sense applied to the destruction of pathogenic blood protozoa by various drugs with a narrow therapeutic index. Consequently the choice of the sulphonamide to be used at present is indicated by considerations of availability and economy rather than toxicity and relative effectiveness. In the ruminant the parenteral route of administration is desirable if not essential (Clark this journal) so that the rate of absorption is of less importance than solubility, stability in fluid form and rate of excretion.

In practice considerable success has been achieved in the treatment of heartwater in ruminants, but to be of maximum value it is essential that treatment be started as early in the course of the disease as possible. From a clinical point of view, symptoms upon which it is possible to base a diagnosis appear so late in the course of the disease that the animal succumbs before sufficient time has elapsed for the specific action of the sulphonamide to become effective. For this reason many failures in treatment have been reported and have been reproduced in carefully controlled experiments under laboratory conditions.

Neitz also observed that uleron administered during the incubation period of the disease had a beneficial effect on the course of the subsequent infection. This effect was shared by the other sulphonamides. A successful method of immunizing adult animals was developed which consisted in infecting animals with infected blood or spleen emulsion and commencing treatment in the early febrile reaction period, before clinical symptoms had developed. Nevertheless the need for immunization of valuable stud animals has stimulated the search for a therapeutic agent more reliable and more effective than the sulphonamides.

Rickettsia ruminantium has been shown to bear some relationship to the lymphogranuloma—psittacosis group of organisms (Rake, Alexander and Hamre, 1945). According to Wong and Cox (1948) aureomycin is an effective therapeutic for this group of pathogens so that the application of the results of their work was indicated.

The results of the experimental work carried out are the basis of this report.

METHODS AND MATERIALS.

Two strains of heartwater virus, known respectively as "Mara" and "Kaalplaas" from the names of the farms from which they were isolated originally, were selected for the work. They have been used extensively for experimental purposes and were selected because they are known regularly to produce a severe clinical disease with a high percentage mortality in sheep.

The virus strains were stored as 10 per cent. spleen emulsion in phosphate buffer in a dry ice cabinet at approximately -74° C. Stored in this way the fragile virus retained full infectivity for long periods but for these experiments sheep were used which had been infected by the intravenous subinoculation of 10 c.c. of fresh citrated blood tapped at what was judged to be the height of the febrile reaction of the donor.

Mature Merino sheep purchased from known heartwater free areas were used and no immune sheep were encountered. The sheep were a uniform group weighing approximately 50 lb. each. Throughout the work they were maintained in a stable on a maintenance ration.

The aureomycin hydrochloride with sodium glycinate was used as a $1\frac{1}{2}$ per cent. aqueous solution administered intrajugularly.

EXPERIMENTAL.

(I) A group of ten sheep was infected with the Mara strain of heartwater. Eight days later, at the commencement of the febrile reaction eight were treated on four successive days with 17 c.c. of $1\frac{1}{2}$ per cent. solution (0.25 gm.) of aureomycin intravenously. The daily dose was calculated as 5 mg. per lb. body weight.

Result.

The eight treated sheep developed only a mild thermal reaction, which lasted about three days. During treatment two appeared to be listless and disinclined to feed. Complete recovery was rapid and uninterrupted. When challenged 11 days later i.e. 22 days after infection, all were immune.

The two untreated sheep showed marked febrile reactions, with clinical symptoms of heartwater. One died on the eleventh day after injection, the other recovered after a prolonged illness.

Conclusion.

The intravenous administration of 5 mg. of aureomycin per lb. on four successive days, commencing as early as possible in the course of the disease, resulted in the prompt recovery of 8 sheep with the development of a solid immunity.

The result of this experiment clearly indicated that aureomycin was an antibiotic of great therapeutic value in the treatment of heartwater. In the interest of economy it was essential to determine the minimum therapeutic dose.

(II) Twelve sheep were infected with the Mara strain of virus. Either on the 9th or 10th day after injection the febrile reaction commenced in all the sheep which were then divided at random into six groups of two each, one pair to serve

as untreated controls, and five pairs to receive 1.0, 0.5, 0.25, 0.125, 0.05 gm. representing a single administration of aureomycin over the range 1 to 20 mg. per lb. body weight. It must be borne in mind that treatment was commenced very early in the course of the reactions before any clinical symptoms other than those of fever had appeared and before it would be possible for a clinician to arrive at any diagnosis.

Result.

1. Dose 1.0 gm. (20 mg. per lb.). By the following morning the temperature of both sheep had returned to normal. No subsequent relapse occurred.

2. Dose 0.5 gm. (10 mg. per lb.). The temperature of one sheep was down to normal the following day and no relapse occurred. The temperature of the other sheep dropped from 106.2° to 104° but fluctuated at a slightly elevated level for twenty days. The sheep was off feed and lost condition but at no time could any pathognomonic symptoms of heartwater be detected. An intercurrent infection was suspected but could not be established with certainty.

3. Dose 0.25 gm. (5 mg. per lb.). The temperature of both sheep was normal the following day. One animal showed a second febrile reaction commencing on the 4th day after treatment and which lasted two days.

4. Dose 0.125 gm. (2.5 mg. per lb.). The temperature of both animals gradually returned to normal over a period of two to three days. The sheep made uninterrupted recoveries although one was disinclined to feed for one day.

5. Dose 0.05 gm. (1.0 mg. per lb.). Although both animals recovered the febrile reaction as compared with the controls was only slightly modified and both were obviously sick, the one for a period of five days.

6. Untreated controls. Both showed the normal febrile reaction of about seven days duration accompanied by clinical symptoms of heartwater. One died and the other recovered.

All the survivors were challenged with homologous virus on the 27th day after infection. All were solidly immune whereas two controls reacted and died.

Conclusion.

It was concluded that:—

1. The minimal therapeutic dose was approximately 2.5 mg. per lb. body weight if a single treatment early in the course of the reaction was to be applied.

2. A dose of 1.25 mg. per lb. had a marked beneficial effect but could not be relied upon to exclude the possibility of a subsequent relapse.

3. A dose of 0.05 mg. per lb. was insufficient to produce more than a slight modification in the course of the disease.

4. All the treated animals even though they received up to 8 times the minimal therapeutic dose developed a solid immunity.

(III) A further experiment was carried out to confirm the previous experiment on another strain of virus, to investigate the result of repeated smaller doses at intervals of 24 hours, and to obtain data on the effect on immunity production

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of larger doses repeated at intervals. The Kaalplaas strain was used to infect 23 sheep. By the 9th day a febrile reaction had commenced in all. With the exception of a single sheep which was given one injection of aureomycin at the rate of 20.0 mg. per lb. weight, pairs of sheep were treated once, twice, or three times as shown in Table 1; two were left as untreated controls.

TABLE 1.

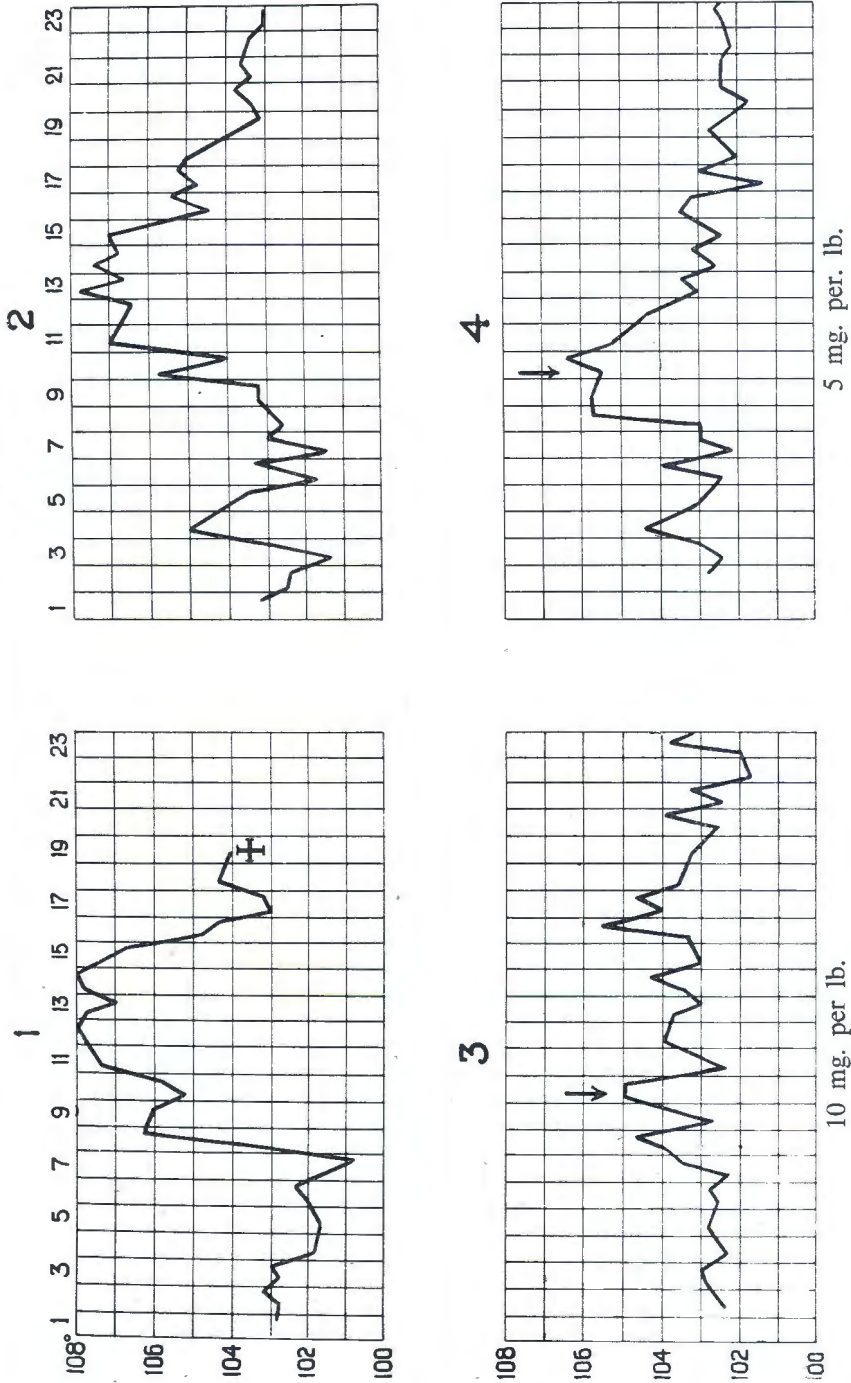
The effect of varying amounts of aureomycin administered to sheep infected with the Kaalplaas strain of heartwater.

Sheep No.	Dose in mg. per lb.	Result.
81623	20.0 × 1	Rapid recovery.
81660	10.0 × 2	Rapid recovery.
79548	10.0 × 2	Rapid recovery.
81553	10.0 × 1	Rapid recovery.
81678	10.0 × 1 (3)	Recovery with subsequent partial relapse.
81569	5.0 × 3	Rapid recovery.
81929	5.0 × 3	Rapid recovery.
81721	5.0 × 2	Rapid recovery.
81928	5.0 × 2	Rapid recovery.
81691	5.0 × 1	Rapid recovery.
81551	5.0 × 1 (4)	Rapid recovery.
80125	2.5 × 3	Rapid recovery.
81357	2.5 × 3	Rapid recovery.
80464	2.5 × 2	Rapid recovery.
79559	2.5 × 2 (5)	Recovery delayed with subsequent slight relapse.
79889	2.5 × 1	Recovery slightly delayed with subsequent relapse.
80618	2.5 × 1 (6)	Similar reaction.
79711	1.0 × 3	Rapid recovery after 3rd treatment.
80449	1.0 × 3 (7)	Rapid recovery after 3rd treatment.
81659	1.0 × 2	Recovery delayed with subsequent relapse.
80446	1.0 × 2 (8)	Recovery considerably delayed and subsequent slight relapse.
81728	Nil (2)	Usual severe febrile reaction with recovery.
81717	Nil (1)	Usual severe reaction and death.

NOTE.—10 × 2 = two injections of 10 mg. per lb.

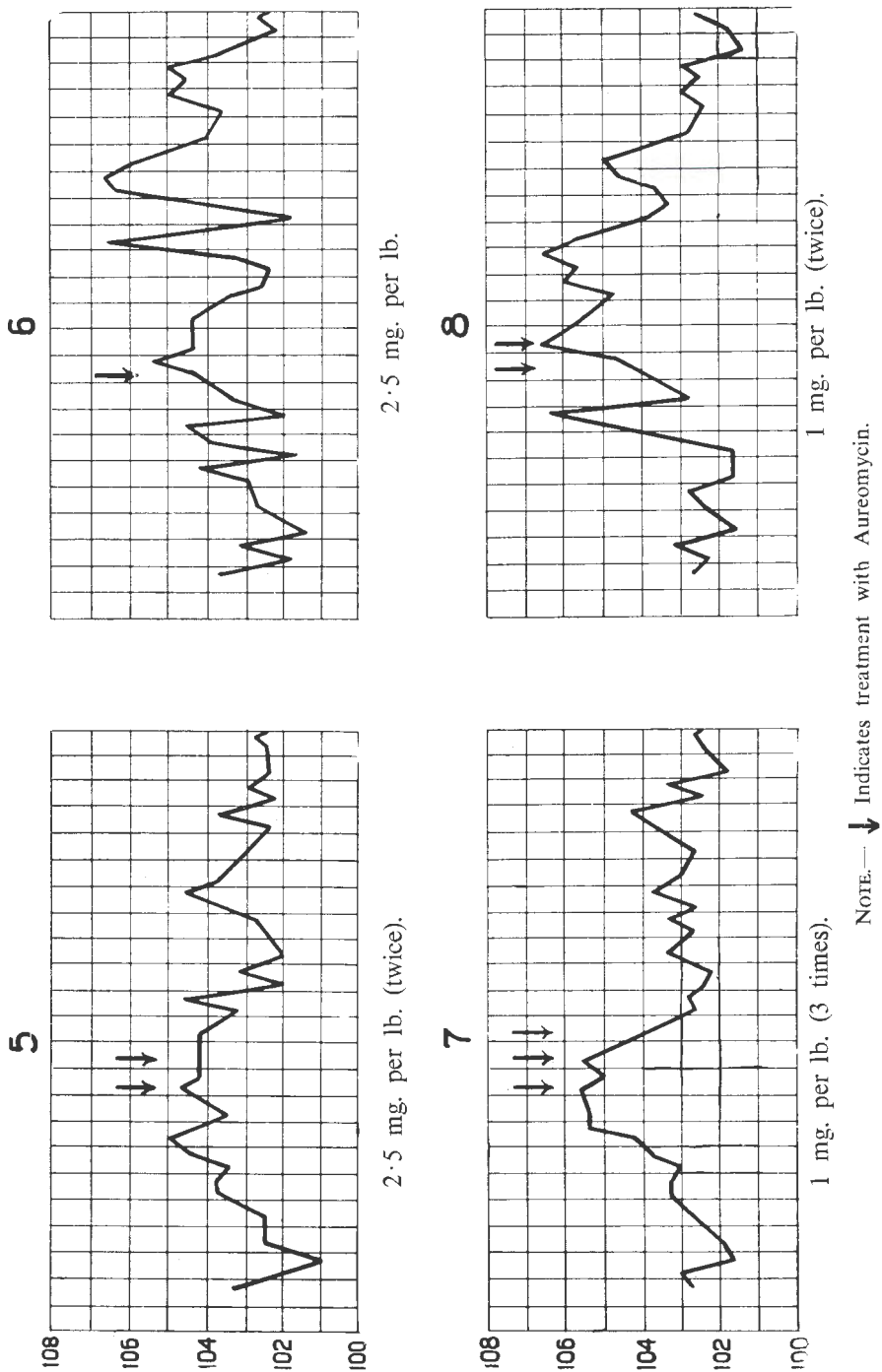
(1) to (8) refers to temperature chart in Fig. 1.

FIG. 1.—Representative Temperature Reactions in Treated and Untreated Sheep.



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FIG. 1.—(Continued.)



Result.

1. The result of prime importance was that all the sheep that were treated with aureomycin recovered even though the dose was as small as 1 mg. per lb. body weight.

2. All the sheep which recovered, whether treated or untreated, were solidly immune to a challenge of the homologous virus 27 days after infection to which two susceptible controls succumbed.

Conclusions and comment.

Although 23 sheep were used in this experiment, the number was too small for any dogmatic conclusions to be drawn. Further it would be reasonable to contend that the apparent beneficial results of treatment might be discounted since, in each of the three experiments described, only one of a pair of untreated controls died, while the other showed the anticipated severe febrile reaction but recovered. It is believed, however, that this was a matter of pure chance, because a considerable experience derived from the routine passage of both these strains of heartwater through hundreds of sheep has shown that a mortality in excess of 75 per cent. can usually be anticipated.

Read in conjunction with the temperature charts in figure 1 the results of the three experiments described above permit a number of highly significant conclusions.

The great value of aureomycin as a therapeutic agent was unquestionable. Administered by intravenous injection early in the course of the febrile reaction a dose of 2.5 mg. per lb. live weight appeared to be the minimal quantity that could be relied upon to control the disease by a single treatment. Since the recovery of one sheep was delayed, and a mild relapse subsequently occurred, even after repetition of this dose after an interval of 24 hours, and in one instance a mild relapse occurred after administering as much as a single dose of 10 mg. per lb., caution should be exercised in concluding that 2.5 mg. per lb. would invariably be effective. In general practice differences in local conditions, in the resistance of individual animals, and differences in the virulence or lethal characteristic of different virus strains will have to receive adequate consideration.

If it is conceded that 2.5 mg. per lb. is the indicated dosage, then it would appear to be immaterial whether that total amount is given as a single dose or in two or three divided doses at intervals of 24 hours. This was supported by the fact that three consecutive treatments with as small an amount as 1.0 mg. per lb. (chart 7) resulted in uninterrupted recovery, while two such treatments (chart 8) were not equally effective. One cannot escape the belief, which is supported by the clinical findings at the time, that if the sheep whose temperature chart is shown in curve 8 had received a third dose at the reduced rate, the downward trend of the curve would have been maintained, and uninterrupted recovery without a relapse would have been the outcome.

Finally when the question of the minimal effective dose is considered it should be appreciated that as small an amount as 1.0 mg. per lb. had a pronounced effect upon the course of the disease. This is the contention of some private veterinarians who have used aureomycin in their practice on our advice; in fact, one such practitioner is emphatic that 1.0 mg. per kilo has been effective. This may well be the case but it is not supported by the experimental evidence submitted above.

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There arises the interesting possibility that in the interests of economy and safety a combination of aureomycin with one of the cheaper sulphonamides might be effective and ultimately the therapeutic method of choice.

As regards the production of immunity it is of considerable importance that even as comparatively large a dose as 20 mg. per lb. either administered as a single injection or in two divided doses did not interfere with the development of solid immunity. This prompted an investigation of the effect of administering a dose of aureomycin in the incubative period of the disease, particularly with a view to developing a safe method of immunizing valuable adult breeding stock.

(IV) Ten sheep were infected with the Kaalplaas strain of virus. On the fifth day i.e. some four or five days before the anticipated start of the febrile reaction four groups of two each were given a single injection of aureomycin over the range 2.5 to 15 mg. per lb. No other treatment was applied. The remaining two sheep served as untreated controls.

Result.

1. 15 mg. per lb.—no febrile or clinical reaction.
2. 10 mg. per lb.—no febrile or clinical reaction.
3. 5 mg. per lb.—one showed no reaction, the other a delayed very mild fever.
4. 2.5 mg. per lb.—reaction slightly delayed and only slightly modified in severity. Both recovered.
5. Untreated controls—both reacted, one died and one recovered.

An immunity test was given on the 23rd day after infection. All the sheep were solidly immune whereas two susceptible controls died.

Conclusion.

Administration of aureomycin during the period of incubation had a marked effect upon the subsequent reaction. It would appear that the effective dose was somewhat higher than the minimal therapeutic dose i.e. between 2.5 mg. and 5 mg. per lb.

The other point of prime importance was that as the protective dose was increased, so the severity of the subsequent reaction decreased until, at a rate of 10 mg. per lb. or higher, the reaction was suppressed completely. Nevertheless a solid immunity was the outcome.

DISCUSSION.

The experiments described have all been connected with the treatment of heartwater commenced in the early stages of the disease in sheep. In the laboratory one or more strains of virus are maintained by serial passage in sheep. Some of these animals became available for treatment at a later stage, when clinical symptoms such as staggering and inco-ordination of movement had appeared. Using repeated doses at a rate of 2.5 mg. per lb., the percentage recoveries was astonishingly high. The great value of the drug as a specific therapeutic agent superior to any other investigated up to the present was established.

Naturally it is of great value to ascertain whether similar results would be obtained in the treatment of cattle. Unfortunately the reaction of susceptible cattle to artificial infection with heartwater is not as constant as in sheep, so that an accurate estimate of the minimal effective dose has not, as yet, been possible. However, some 15 animals and several clinical cases of naturally contracted heartwater, confirmed by subinoculation into sheep, have been treated at the rate of 2.0 to 2.5 mg. per lb. All recovered and it is worthy of note that two were recumbent when treatment was commenced. At this stage, administration of sulphonamides even in massive doses, is ineffective, yet both animals recovered, the one after a single injection, the other after three treatments.

From these results it would appear that the action of the drug was rickettsiastatic rather than rickettsiacidal, because it is believed that a solid immunity develops only if infection has been established, though controlled, and the defence mechanism of the body has been organized to control multiplication of the parasite. In this connection it should be remembered that neither a massive curative dose nor a massive prophylactic dose administered during the incubation period adversely affected immunity production. Further the action of the drug was cumulative, since it was immaterial whether the minimal effective quantity was given in a single or in divided doses.

After this work had been completed, Smadel *et al.* (1951) reported the results of their investigations on immunization against scrub typhus by combined living vaccine and chemoprophylaxis. The similarity in the final results are immediately apparent.

SUMMARY.

1. The value of aureomycin in the treatment of heartwater has been established. It is considerably more effective than the sulphonamides.
2. The minimal therapeutic dose for sheep appears to be 2.5 mg. per lb. administered intravenously either as a single dose or in divided doses at intervals of 24 hours.
3. Doses smaller than the curative dose have a marked effect upon the course of the disease.
4. In cattle even advanced cases have responded promptly to administration of 2 to 2.5 mg. per lb. It was possible that doses as small as 1 mg. per lb. were effective.
5. Administered in the incubation stage of the disease in amounts approximately double the curative dose it had a marked effect upon the subsequent course of the disease.
6. Prophylactic or curative administration in doses up to 20 mg. per lb. did not interfere with immunity production.
7. Aureomycin alone or in combination with a sulphonamide is suggested at present as the drug of choice for treatment, or together with live virus for immunization.

This work was facilitated by the supply in very generous quantities of aureomycin hydrochloride with sodium glycinate by the Director, Lederle Laboratories, Pearl River, N.Y., U.S.A., to whom we wish to take this opportunity of expressing our indebtedness.

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REFERENCES.

- NEITZ, W. O. (1939). Die Wirkung von Uleron auf das Herzwasser (*Rickettsia ruminantium*) der Schafe. *Berl. u. Münch. Tierärztl. Wschr.*, Jg. 1939, Nr. 9, pp 134-135.
- NEITZ, W. O. AND ALEXANDER, R. A. (1941). The immunization of calves against heartwater. *Jnl. South African Vet. Med. Assoc.*, Vol. 12, No. 4, pp. 103-111.
- NEITZ, W. O. AND ALEXANDER, R. A. (1945). Immunization of cattle against heartwater and the control of the tick-borne diseases, redwater, gallsickness and heartwater. *Onderstepoort Jnl.*, Vol. 20, pp. 137-158.
- RAKE, G., ALEXANDER, R. A. AND HAMRE, DOROTHY M. (1945). The relationship of the agent of heartwater fever—*Rickettsia ruminantium*. *Science* Vol. 102, No. 2652, pp. 424-425.
- SMADEL, J. E., LEY, H. L., DIERCKS, F. H., TRAUB, R., TIPTON, V. J. AND FRICK, L. P. (1951). Immunization against scrub typhus. I. Combined living vaccine and chemoprophylaxis in volunteers. *Am. J. of Hygiene*, Vol. 53, No. 3, pp. 317-331.
- WONG, S. G. AND COX, H. R. (1948). Action of Aureomycin against experimental Rickettsial and viral infections. *Ann. New York Acad. Sc.*, Vol. 51, p. 290.