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PROMISING THERAPEUTIC AGENTS FOR THE ELIMINATION OF *ANAPLASMA MARGINALE* IN THE CARRIER ANIMAL

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The need for and the implementation of an effective program for the reduction of losses due to anaplasmosis has been discussed for years by both the veterinarian and the livestock producer. The continuation of research progress inevitably brings the goal of anaplasmosis control nearer. In recent years significant progress has been made in the area of vaccine production[3, 11, 14] and in the development of new and effective diagnostic procedures[1]. Both of these advances can perform an important function in the reduction of animal losses due to anaplasmosis. The persistant nature of *Anaplasma* infections, even after apparent recovery, creates serious problems when eradication programs are considered. A therapeutic approach which would effectively eliminate the disease agent from infected animals will probably play an important role in control programs of the future.

For many years the tetracyclines have been the drugs of choice in treating clinical anaplasmosis and in eliminating the carrier state of infection. A number of workers[4, 6, 7, 13] have in the past reported on the efficacy of these drugs showing them to be useful and effective. The elimination of the carrier status with tetracyclines usually requires either daily injections for 10 or more days or a low level feeding program for 30 to 60 days. Both procedures are time consuming and expensive. A more efficient regime to eliminate carrier infections would significantly contribute to the control of anaplasmosis.

A program of test and treatment appears practical for the eradication of anaplasmosis in some areas of the country[15]. The problem of eradication is more difficult in those areas where ticks serve as biological vectors and wildlife are a reservior of infection.

During the past few years a new drug, in addition to the tetracyclines, has attracted some attention as a possible therapeutic agent. In 1965 the effectiveness of an a-dithiosemicarbazone (356 C 61)* in the treatment of anaplasmosis, was reported[2]. Since that time serveral reports have been published confirming the effectiveness of this agent[5, 9, 10, 12]. More recently favorable results have been reported using the a-dithiosemicarbazone with oxytetracycline**[8]. The results suggest that the simultaneous use of these drugs was more effective than either alone.

In addition to the dithiosemicarbazone a second material, Imidocarb (3, 3'-Bis-(2-imidazolin-2-yl) - carbanilide dihydrochloride) or 4A65,* has been tested

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and shows promise in the treatment of anaplasmosis.

This report will review results of studies dealing with these 3 agents, oxytetracycline, 356 C 61, and 4A65, when used for the elimination of anaplasmosis carrier status in splenectomized calves.

Splenectomized calves have proven useful in these preliminary studies largely because of the characteristic *A. marginale* relapses which occur in treated animals that retain a non-apparent infection. In these experiments, calves failing to show a relapse within 62 days of treatment have been shown to be free of infection by subsequent infectivity tests, and in many instances by later re-challenge. Splenectomized calves are an abnormal system, and these results should not stand alone without further trials in intact cattle. These results are useful to delineate the relative activity of various treatment schedules, and provide the basis for furture studies.

MATERIALS AND METHODS

Treatment was administered to 59 splenectomized, *A. marginale*-infected dairy type calves of mixed breeding ranging in age from 5-21 months with an average of 10.8 ± 4 months.

Groups I, II, and III consisted of 14 calves treated with 356 C 61 only (Table 1). Calves in Group I were treated 5 times at 24 hour intervals with 5mg/kg injected intravenously (I.V.). Calves in Group II were treated 10 times at 24 hour intervals with 5 mg/kg injected I.V. A total of 8 calves in Group III were injected intramuscularly (I.M.) either 3 or 4 times with 5 mg/kg 356 C 61 at varying intervals. Treatment was administered to 1 calf, 3 times, at 48 hour intervals; to 3 calves, 4 times, at 3 and 4 day intervals (twice a week); to 3 calves, 3 times, at 7 day intervals; and to 1 calf, 3 times, at 14 day intervals.

Groups IV, V, VI, VII, and VIII consisted of 33 calves treated with 356 C 61 oxytetracycline (Table 2). Calves of Group IV were treated 3 times at either a 24 or 48 hour interval with 2 mg/kg 356 C 61 and 11 mg/kg oxytetracycline. Calves of Group V were treated 3 times at either a 24 or 48 hour interval with 5 mg/kg 356 C 61 and 11 mg/kg oxytetracycline. Calves of Group VI were treated 3 times at 72 hour intervals with 5 mg/kg 356 C 61 and 11 mg/kg oxytetracycline. Calves of Group VII were treated 3 times at either 24 or 48 hour intervals with 10 mg/kg 356 C 61 and 11 mg/kg oxytetracycline. Calves of Group VIII were treated 2 times at either a 24 or 48 hour interval with 5 or 10 mg/kg 356 C 61 and 11 mg/kg oxytetracycline. The 356 C 61 and oxytetracycline were mixed in and diluted with 150 ml 0.85% NaCl (PSS) and injected I.V.

Groups IX and X consisted of 12 calves treated with 4A65 alone and in combination with 356 C 61 and oxytetracycline (Tables 3 and 3A). Calf 405 was treated 1 time with 4 mg/kg 4A65, 15 mg/kg 356 C 61 and 44 mg/kg oxytetracycline. The 4A65 was injected I.M., and the 356 C 61 and oxytetracycline were diluted in PSS and injected I.V.

Calves 135 and 219 were each treated 1 time, with 6 mg/kg 4A65 I.M., and 15 mg/kg 356 C 61 I.V. Calves 424 and 431 were each treated 1 time with 15 mg/kg 4A65 I.M. (Table 3).

Calves 217 and 248 were each treated 3 times at 24 hour intervals with 2 mg/kg 4A65 I.M. and 5 mg/kg 356 C 61 I.V. Calf 450 was treated 3 times at 24 hour intervals with 5 mg/kg 4A65 I.M., and 2 mg/kg 356 C 61 I.V. Calf 245 was treated

3 times at 24 hour intervals with 4 mg/kg 4A65 I.M. Calves 405 and 413 were each treated 3 times at 24 hour intervals with 5 mg/kg 4A65 I.M. Calf 265 was treated 3 times at 24 hour intervals with 6 mg/kg 4A65 I.M. (Table 3A).

All animals were monitored for changes in percent parasitemia, packed cell volume (PCV), and serum complement-fixation titers for anaplasmosis. Blood samples were collected for this purpose twice a week a minimum of 14 days before and 60 days after treatment. The relapse period is expressed as days following treatment required for the development of a 1% or greater *A. marginale* parasitemia. Except for 3 calves in Table 3A, infectivity trials were conducted on all aminals, not showing an *A. marginale* relapse, by an I.V. injection of 200 ml of whole blood from the treated animal into a susceptible splenectomized calf. With the exception of Group I and II the time at which infectivity trials were conducted was never less than 60 days and on an average was 87 days. Groups I and II were tested for infectivity 5 to 20 days after treatment. In some instances blood from 2 and 3 calves was pooled and injected into 1 splenectomized calf.

RESULTS

The results following the use of 356 C 61 alone was presented in Table 1. Treatment with 5 mg/kg of 356 C 61 for 5 consecutive days failed to eliminate the *A. marginale* infection. The use of 5 mg/kg 356 C 61 injected 3 and 4 times at 2, 3-4, 7 and 14 day intervals was equally unsuccessful. Treatment for 10 consecutive days with 5 mg/kg 356 C 61 was apparently successful in removing evidence of *A. marginale* based on infectivity tests, but resulted in fatal toxicosis in every animal.

The results of combined therapy with 356 C 61 and oxytetracycline are presented in Table 2. Group IV, using only 2 mg/kg 356 C 61, in combination with a constant of 11 mg/kg oxytetracycline, failed to prevent an *A. marginale* relapse. Groups V and VII, using 5 or 10 mg/kg 356 C 61 in combination with oxytetracycline for 3 times at either a 24 or 48 hour interval eliminated evidence of infection based on the absence of a relapse and negative infectivity trails. Group VI given similar drug levels 3 times at 72 hour intervals was less effective, and 1 of the 3 treated calves developed an *A. marginale* relapse 39 days after the last treatment. Group VIII given only 2 injections of similar drug levels at either 24 or 48 hour interval failed in every case to prevent relapse infection.

The results of 1 injection of 4A65 alone and in combination with 356 C 61 and oxytetracycline are presented in Table 3. All calves receiving only 1 injection, showed a relapsing infection an average of 42 ± 7 days after treatment.

The results of 3 injections at 24 hour intervals of 4A65 alone and in combination with 356 C 61 are presented in Table 3A. In every instance evidence is present to indicate an elimination of *A. marginale*. Infectivity trials are complete in 4 of 7 calves, and the 3 remaining animals have shown no sign of relapse for over 60 days.

DISCUSSION AND CONCLUSIONS

A combination of oxytetracycline and 356 C 61 appears more effective than either given alone. It would appear from these results that this action represents more than just an additive effect, and that there may be a synergistic effect in this instance. Using the drug levels reported, 4A65 appears equally as competent to eliminate infection when given alone or in combination with 356 C 61. This drug

when given I.V. was responsible for excessive salivation, lacrimation and labored breathing. These side effects appear less severe when the drug is administered I.M. or S.C., and the efficacy was not altered.

Relapses when they occur, usually do so between 30-45 days. The longest relapse period observed to date has been 62 days. The occurrence of a diagnostic relapse negates the need for infectivity trials. Our experience has been that treated calves, failing to show a relapse within a 62 day period are consistently free of infection as determined by infectivity trials.

These studies suggest that marked improvements are possible in treatment techniques, however much needs to be done before these drugs can be freely used and dispensed. Toxic side effects, tissue residues, as well as trials in intact animals are indicated. Hopefully such trials will result in the evolution of more effective and efficient drugs for the elimination of infection.

SUMMARY

Two new drugs, a-dithiosemicarbazone (356 C 61) and 3, 3'-Bis (2-imidazolin-2-yl) - carbanilide dihydrochloride (4A65) have been successfully used to treat splenectomized calves with anaplasmosis. Carrier infections were eliminated with 5 or 10 mg/kg 356 C 61 and 11 mg/kg oxytetracycline when given 3 times at either a 24 or 48 hour interval. In addition 5 mg/kg 356 C 61 plus 2 mg/kg 4A65 given 3 times at 24 hour intervals was effective in eliminating *A. marginale* infections. Levels of 4 and 6 mg/kg of 4A65 given 3 times at 24 hour intervals has proven successful in eliminating *A. marginale* infection.

Table 1

Use of 356 C 61 to Eliminate Carrier Status in Splenectomized Calves

	No. of Animals	Dose Rate mg/kg	Route of Inoculation	Number of Inoculations	Interval of Inoculations	Results of Treatment	Infectivity Trials
Group I	3	5	I.V.	5	24 hrs	Failed	Positive
Group II	3	5	I.V.	10	24 hrs	Successful	Negative *
Group III	8	5	I.M.	3 and 4	2, 3-4, 7 and 14 days	Failed Relapse: 40 ± 11 ds	N.T.

* All calves treated with 356 C 61 - 10 times at the 5 mg/kg level died, apparently due to drug toxicity.

N.T. - No Test I.V. - Intravenous I.M. - Intramuscular

Table 2

Use of 356 C 61 in Combination With Oxytetracycline to Eliminate *Anaplasma* Carrier Status of Splenectomized Calves

	No. of Animals	Dose Rate mg/kg		Route of Inoc.	No. of Inoc.	Interval of Inoc.	Results of Treatment	Relapse Time Days	Infectivity Trials
		356 C 61	Oxytet.						
Group IV	3	2	11	I.V.	3	24 or 48 hrs	3/3 Failed	31.7 ± 14.1	N.T.
Group V	11	5	11	I.V.	3	24 or 48 hrs	11/11 Successful	--	11/11 Negative
Group VI	3	5	11	I.V.	3	72 hrs	2/3 Successful	39	2/3 Negative
Group VII	8	10	11	I.V.	3	24 or 48 hrs	8/8 Successful	--	8/8 Negative
Group VIII	8	5 or 10	11	I.V.	2	24 or 48 hrs	8/8 Failed	41.9 ± 9.6	N.T.

I.V. - Intravenous N.T. - No Test

Table 3

Use of 4A65 Alone and in Combination With 356 C 61 to Eliminate The Anaplasma Carrier Status of Splenectomized Calves

Group IX

Calf Number	4A65 mg/kg	4A65 Route	4A65 No. of Inoc.	356 C 61 mg/kg	356 C 61 Route	356 C 61 No. of Inoc.	Result of Treatment	Relapse Time In Days	Infectivity Trial
405 *	4	I.M.	1	15	I.V.	1	Failed	42	N.T.
135	6	I.M.	1	15	I.V.	1	Failed	54	N.T.
219	6	I.M.	1	15	I.V.	1	Failed	43	N.T.
424	15	S.C.	1	--	--	--	Failed	38	N.T.
431	15	S.C.	1	--	--	--	Failed	35	N.T.
								(42 ± 7) Days	

* Treated simultaneously with 44 mg/kg oxytetracycline I.V.

I.M. - Intramuscular I.V. - Intravenously N.T. - No Test S.C. - Subcutaneously

Table 3A

Multiple Injections of 4A65 Alone and in Combination With 356 C 61 to Eliminate The Anaplasma Carrier Status of Splenectomized Calves

Group X

Calf Number	4A65 mg/kg	4A65 Route	4A65 No. of Inoc.	4A65 Interval	356 C 61 mg/kg	356 C 61 Route	356 C 61 No. of Inoc.	356 C 61 Interval	Results of Treatment	Relapse Time In Days	Infectivity Trial
217	2	I.M.	3	24 hrs	5	I.V.	3	24 hrs	Successful	>120	Negative
248	2	I.M.	3	24 hrs	5	I.V.	3	24 hrs	Successful	>120	Negative
450	5	I.M.	3	24 hrs	2	I.M.	3	24 hrs	Successful	>60	N.T.
245	4	I.M.	3	24 hrs	--	--	--	--	Successful	>120	Negative
405	5	I.M.	3	24 hrs	--	--	--	--	Successful	>90	N.T.
413	5	I.M.	3	24 hrs	--	--	--	--	Successful	>90	N.T.
265	6	I.M.	3	24 hrs	--	--	--	--	Successful	>120	Negative

I.M. - Intramuscular I.V. - Intravenous N.T. - No Test

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