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THE CHEMOTHERAPEUTIC EFFICACY OF IMIDOCARB DIHYDROCHLORIDE ON CONCURRENT BOVINE ANAPLASMOSIS AND BABESIOSIS

I. The Effects of a Single Treatment

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SUMMARY

The chemotherapeutic efficacy of imidocarb dihydrochloride (3,3'-bis(2-imidazolin-2-yl) carbanilide dihydrochloride) administered as single intramuscular doses of 1.0, 2.0 and 2.5 mg/kg, against concurrent bovine anaplasmosis and babesiosis, is reported. Dosages of 2.0 and 2.5 mg/kg of imidocarb dihydrochloride rapidly inhibited acute ascending concurrent parasitaemias of *Anaplasma marginale*, *Babesia bigemina* and *Babesia argentina*; however, 1.0 mg/kg had a minimal effect on *A. marginale* but was very effective against *B. bigemina* and *B. argentina*. Imidocarb dihydrochloride at 1.0, 2.0 and 2.5 mg/kg inhibited the development of immunity of the acute *Babesia* spp. infections, making the calves more susceptible to babesiosis upon challenge. The inhibition of *A. marginale* parasitaemias was directly related to increasing doses of imidocarb dihydrochloride; however, recrudescing and persisting post-treatment parasitaemias also occurred more frequently at higher doses.

INTRODUCTION

Schmidt, Hirt and Fischer (1969), described the babesicidal effects of the basically substituted carbanilides on *Babesia rodhaini* in mice, of which 3,3'-bis(2-imidazolin-2-yl) carbanilide was the most effective. Beveridge (1969), compared 3,3'-bis(2-imidazolin-2-yl) carbanilide dihydrochloride¹ (IMDC) with three other babesicides against *B. rodhaini* infections in rats and mice and demonstrated that IMDC had the greatest babesicidal effect. Callow and McGregor (1970), demonstrated the sterilising, chemotherapeutic and chemoprophylactic effects of IMDC against *Babesia bigemina* and *Babesia argentina* infections of cattle. Brown and Berger (1970), further described the high degree of chemotherapeutic efficacy of IMDC for *B. bigemina* infections of cattle. Wood (1971), reviewed and summarised the activity of IMDC against *Babesia* infections of cattle. Hart, Roy-Smith, Berger, Simpson and McHardy (1971), demonstrated the efficacy of IMDC against *Anaplasma marginale* in addition to *B. bigemina* and *B. argentina* infections of cattle. Kuttler (1971) and Roby (1972), subsequently showed that IMDC and imidocarb dipropionate eliminated *A. marginale* from carrier cattle. Roby (1972), further substantiated the inhibitory effect of IMDC on acute anaplasmosis in splenectomised calves.

This paper describes the results of chemotherapeutic experiments with IMDC against blood-borne concurrent *B. bigemina*, *B. argentina* and *A. marginale* infections of intact cattle.

MATERIALS AND METHODS

Male Holstein-Friesian calves 300 ± 18 days old, born and raised in a *Boophilus microplus* and babesiosis free Bogotá savanna (altitude 2,600 m), were used in the chemotherapeutic

¹ Imidocarb, Burroughs-Wellcome & Co. Ltd., London, England.

trials. The investigation was performed in tick-free quarters at the Instituto Colombiano Agropecuario, Laboratorio de Investigaciones Medicas Veterinarias located in Bogotá, Colombia. Fifty ml aliquots of blood from each of 18 calves were injected intravenously into a splenectomised calf to give an indication of the absence of latent haemoparasitic infections. In addition, Giemsa-stained combination thin and thick blood films, determinations of complement fixing antibodies for *B. bigemina*, *B. argentina* and *A. marginale* and wet mounts of blood were performed four times before inoculation to further indicate that each of the 18 calves were free of most haemoparasites, particularly anaplasmosis and babesiosis.

Eighteen calves were inoculated subcutaneously with 5 ml of blood containing 2×10^9 *A. marginale* marginal bodies per cm^3 on day -16 and on day -5 the calves were again inoculated subcutaneously with 5 ml of blood containing a mixture of 2×10^9 *B. bigemina* and *B. argentina*. The parasites were identified by examination of Giemsa-stained thin blood films. The number of parasites was estimated by determining in quadruplicate the percentage of organisms in Giemsa-stained thin blood films and multiplying the average percentage of parasitaemia by the average total erythrocyte count per cubic centimetre, also determined in quadruplicate. The haemoparasites were isolated from a splenectomised calf, which was exposed to a natural environment where *Boophilus microplus* ticks predominated in a pasture of the Turipaná Instituto Colombiano Agropecuario experimental farm located at Montería, Córdoba, Colombia (altitude 24 m). The isolates were twice passaged by intravenous inoculation of 100 ml of blood into intact calves to check the purity of the isolates prior to inoculation.

Imidocarb dihydrochloride salt was dissolved in sterilized, distilled water to make a 5 per cent w/v solutions. The drug was injected once intramuscularly on day 0 into the six calves of Group I at 1 mg/kg, the five calves of Group II at 2 mg/kg and the five calves of Group III at 2.5 mg/kg. The two calves of Group IV remained as untreated controls.

The 14 surviving calves were challenged on day 81 post-treatment (PT) by injecting subcutaneously 10 ml of blood containing 3×10^9 homologous *A. marginale* bodies and on day 84 PT, by injecting subcutaneously 10 ml of blood containing a mixture of 9×10^7 homologous *B. bigemina* and *B. argentina*.

The interval of collection of blood and serum samples was as depicted in Figs. 1, 2, 3 and 4. Blood samples were collected from the jugular vein using 1.2 g/litre of disodium ethylenediamine tetra-acetate as anticoagulant. The packed cell volumes (PCV) were determined by the microhaematocrit method after spinning for 5 minutes at 11,500 rev/min in a microhaematocrit centrifuge. Combination thin and thick Giemsa-stained blood films were processed according to a modified method of Mahoney and Saal (1961). Parasitaemias for *A. marginale* and *Babesia* spp. were ascertained by examining the thick and thin blood films and estimates of parasitaemias were made by examining a transect (75 fields using a $\times 100$ oil immersion objective with a $\times 10$ oculars), approximately 3-6 mm from tail of the thin blood films.

All complement-fixation (CF) serum antibody titres were performed by the microtitre method described by Hidalgo and Dimopoulos (1967). *Anaplasma marginale* CF antibody titres were determined by a microprocedural adaptation of a standard method (Anonymous, 1958), using the standard United States Department of Agriculture, Agriculture Research Service antigen. *Babesia bigemina* and *B. argentina* CF antibody titres were ascertained by a microprocedural adaptation of the method described by Mahoney (1962), using distilled water suspensions and extracts of parasitised bovine erythrocytes as antigens according to the method of Mahoney (1967).

RESULTS

Figures 1, 2, 3 and 4 show the results of the subcutaneous inoculation with *A. marginale*, *B. bigemina* and *B. argentina* in 18 susceptible calves; the intramuscular treatment with 1.0, 2.0, 2.5 and 0.0 mg/kg of IMDC; and subsequent homologous subcutaneous challenge, as assessed by packed cell volumes, parasitaemic levels and CF antibody titres. Each point on the curves of the graphs represents the mean and the bar through each point indicates one standard deviation for a given day.

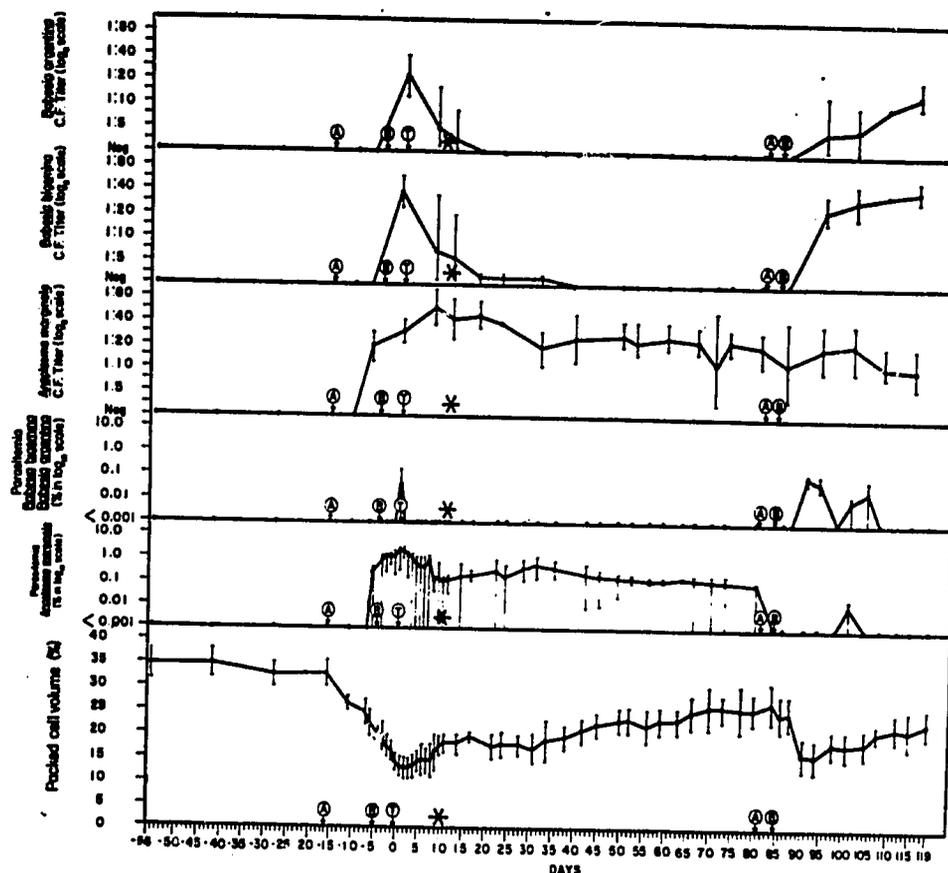


FIG. 1. Haematologic and serologic results of six calves with concurrent anaplasmosis and babesiosis treated intramuscularly with 1.0 mg/kg of imidocarb dihydrochloride. The mean and one standard deviation are represented for each given day. * = death of one calf, A = *Anaplasma marginale* inoculation, B = *Babesia bigemina* and *Babesia argentina* inoculation, and T = day of treatment.

Parasitaemias of *A. marginale* and *Babesia* spp. preceded the lowest PCV values of the four groups by 1 to 3 days. These lowest values occurred on days 3 and 4 PT. The PCV of the treated and untreated groups never returned to pre-inoculation levels during the period of 81 days PT. The slow rate of recovery of the PCV of all groups was accompanied by a persistent variable *A. marginale* parasitaemia. The PCV of the treated groups of calves decreased markedly following challenge on days 81 and 84 PT and was concurrent with a *Babesia* spp. parasitaemia. The surviving untreated control calf had no decrease in PCV.

Imidocarb dihydrochloride at 1.0, 2.0 and 2.5 mg/kg reduced the *A. marginale* parasitaemia to 1.0 per cent or less within an average of 2.3 days PT while untreated controls had a persistent parasitaemia of more than 1 per cent for at least 7 days PT. Dosages of two and 2.5 mg/kg reduced the *A. marginale* parasitaemia to less than 0.1 per cent within three and four days PT respectively but 1 mg/kg failed to do so. A slight increase in *A. marginale* parasitaemia occurred following challenge.

The *Babesia* spp. parasitaemias were not demonstrable after day 1 PT in any of the treated calves but the untreated control calves had a parasitaemia that persisted 11 days. *Babesia* spp. parasitaemias developed in treated and untreated calves following

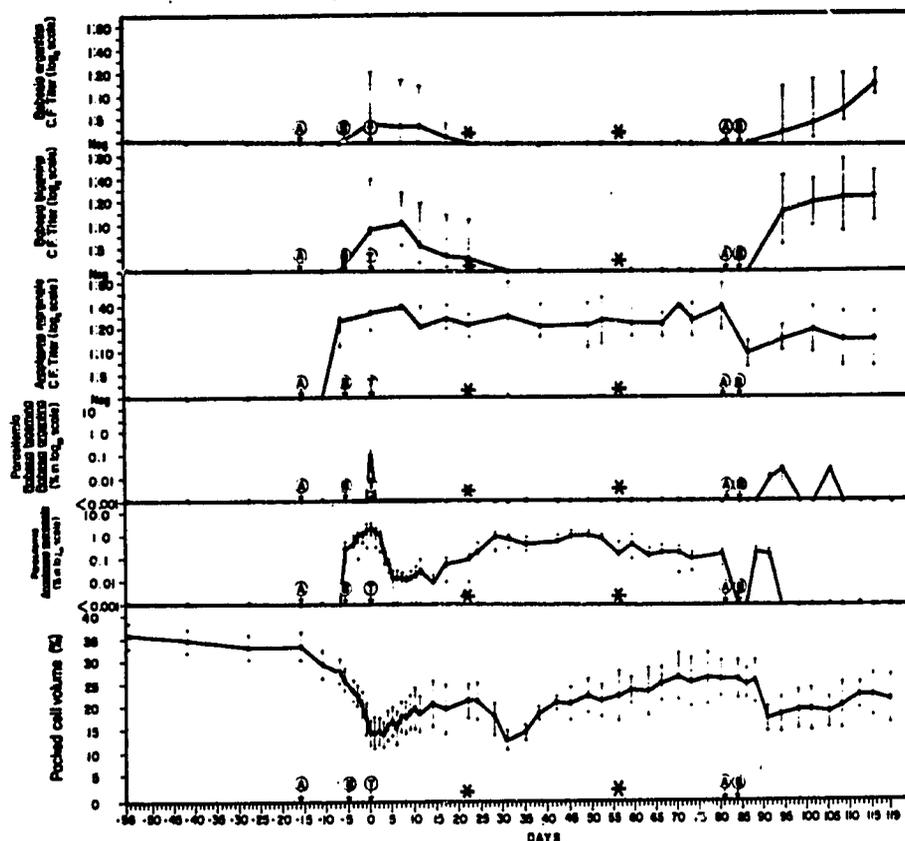


FIG. 2. Haematologic and serologic results of five calves with concurrent anaplasmosis and babesiosis treated with 2.0 mg/kg of imidocarb dihydrochloride. The mean and one standard deviation are represented for each given day. * = death of one calf, A = *Anaplasma marginale* inoculation, B = *Babesia bigemina* and *Babesia argentina* inoculation, and T = day of treatment.

homologous challenge on day 84 PT; however, the treated calves had higher and more persistent parasitaemias with one mortality occurring in the group treated with 2.5 mg/kg.

All calves had positive *A. marginale* CF antibody titres in accordance with the recrudescent and persisting parasitaemias. A slight increase in *A. marginale* CF antibody titres occurred following homologous challenge on day 81 PT.

All calves developed positive *B. bigemina* and *B. argentina* CF titres by day 0. The untreated calves had persistently positive CF titres for *B. bigemina* and *B. argentina* but all of the treated calves developed negative titres for *B. argentina* and *B. bigemina* within 22 and 35 days PT respectively. Calves treated with 2.0 and 2.5 mg/kg had negative *B. bigemina* CF titres before calves treated with 1.0 mg/kg. *Babesia bigemina* and *B. argentina* CF titres of all surviving treated calves became positive within 10 to 17 days after homologous challenge on day 84 PT. The surviving untreated control calf had a persistent positive CF antibody titre for *B. bigemina* and *B. argentina* with a minimal response after challenge.

The five mortalities that occurred during the experiment are described as follows: Group I—one calf died of anaplasmosis on day 10 PT with a 10 per cent PCV and 1.3 per cent *A. marginale* and negative *Babesia* spp. parasitaemias. Group II—one

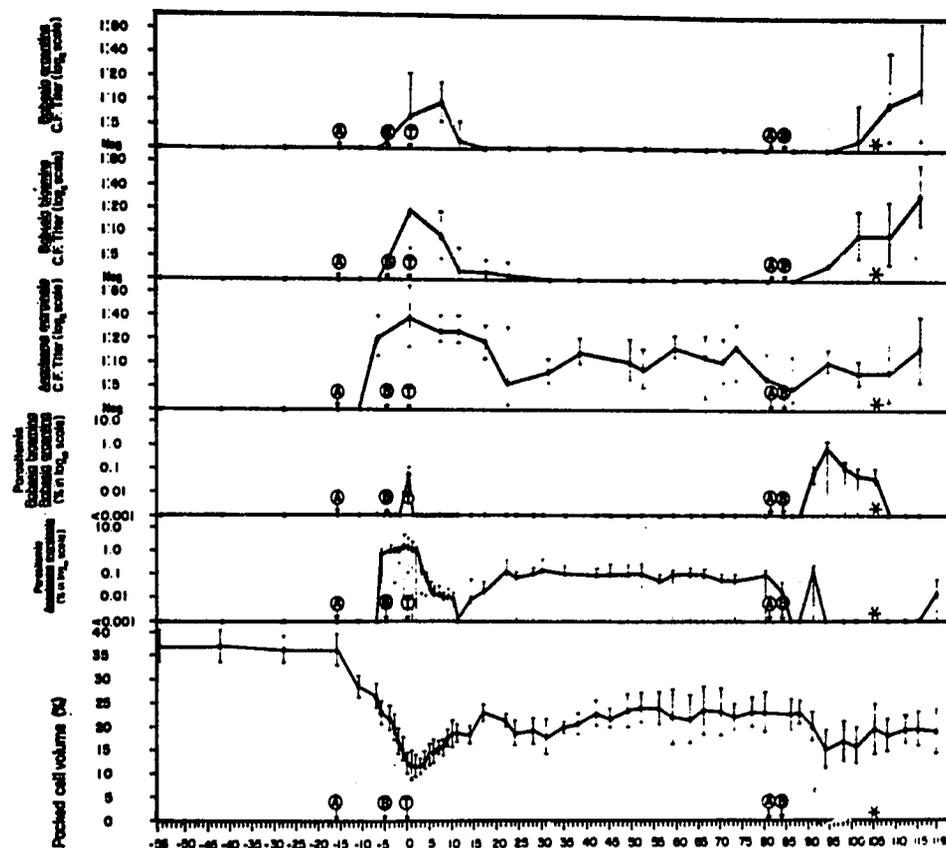


FIG. 3. Haematologic and serologic results of five calves with concurrent anaplasmosis and babesiosis treated intramuscularly with 2.5 mg/kg of imidocarb dihydrochloride. The mean and one standard deviation are represented for each given day. * = death of one calf, A = *Anaplasma marginale* inoculation, B = *Babesia bigemina* and *Babesia argentea* inoculation, and T = day of treatment.

calf died of chronic relapsing anaplasmosis on day 21 PT with a 12 per cent PCV and 0.05 per cent *A. marginale* and negative *Babesia* spp. parasitaemias. Another calf died of chronic relapsing anaplasmosis on day 56 PT with a 10 per cent PCV and 2.3 per cent *A. marginale* and negative *Babesia* spp. parasitaemias. Group III—one calf, challenged on day 84 PT, died of acute babesiosis on day 105 PT with a 12 per cent PCV and 5 per cent *Babesia* spp. and negative *A. marginale* parasitaemias. Group IV—one control calf died of concurrent anaplasmosis and babesiosis on day 4 with a 10 per cent PCV and 4 per cent *A. marginale* and 3 per cent *Babesia* spp. parasitaemias.

A very slight local inflammatory response occurred and disappeared within 3 days at the site of inoculation of the dosages used. Mild transitory dyspnoea, excessive salivation and lachrymation occurred and subsided within 30 minutes post-treatment. The average daily weight gains of surviving calves in Groups I, II, III and IV were 271 ± 70 , 142 ± 41 , 254 ± 49 and 199 g/day respectively from day 0 to day 119 PT.

DISCUSSION

Imidocarb dihydrochloride at 1.0, 2.0 and 2.5 mg/kg rapidly reduced the *Babesia* spp. parasitaemias indicating a high degree of chemotherapeutic effectiveness. These

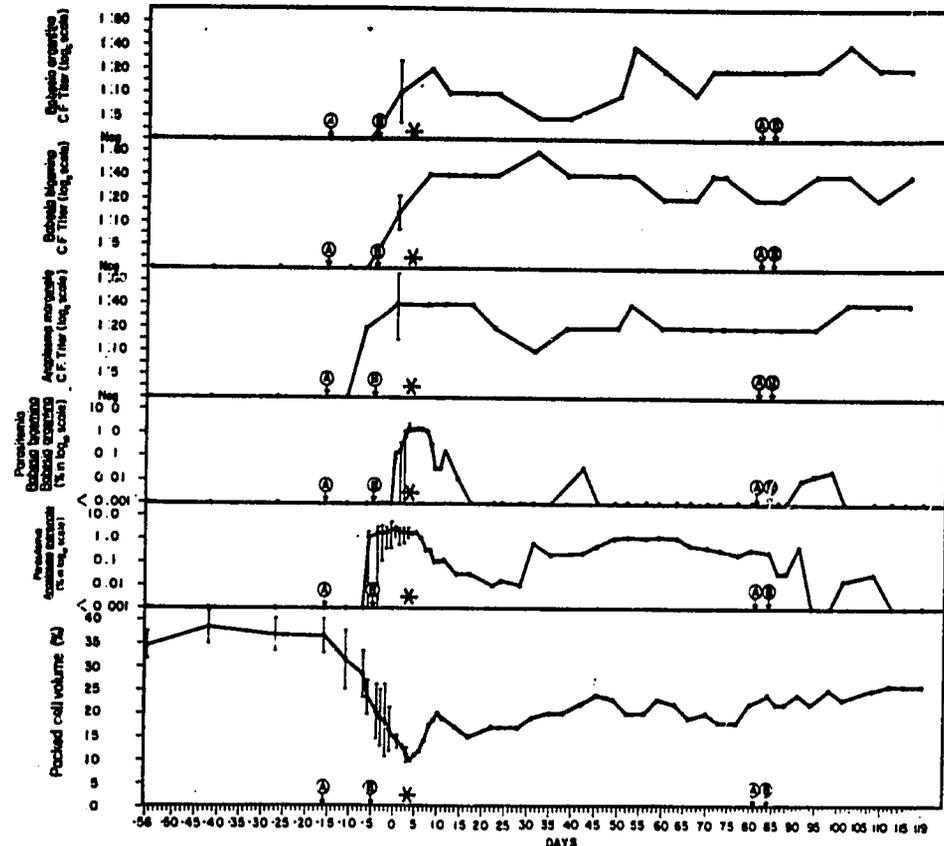


FIG. 4. Haematologic and serologic results of two untreated calves with concurrent anaplasmosis and babesiosis. The mean and one standard deviation are represented for each given day. * = death of one calf, A = *Anaplasma marginale* inoculation, and B = *Babesia bigemina* and *Babesia argentina* inoculation.

findings closely parallel those of Callow and McGregor (1970) and Brown and Berger (1970). The absence of *Babesia* spp. parasitaemias and the development of negative CF titres for *B. bigemina* and *B. argentina* following treatment are highly suggestive that the *Babesia* spp. of the treated calves were eliminated. The death of one treated calf, due to acute babesiosis following challenge on day 84 PT and the positive challenge response of the other treated calves to homologous *Babesia* spp., further indicate that IMDC apparently sterilised the *Babesia* infections. Callow and McGregor (1970), demonstrated that 0.6 mg/kg of IMDC sterilised *B. bigemina* infections and 2.0 mg/kg of IMDC sterilised *B. argentina* infections. Hart *et al.* (1971) and Berger quoted in Brown and Berger (1970), showed that 5.0 mg/kg and 0.75 mg/kg of IMDC respectively sterilised *B. bigemina* infections. Our results indicate that 1, 2 and 2.5 mg/kg of IMDC apparently sterilised acute ascending parasitaemias of *B. bigemina* and *B. argentina*, making the calves more susceptible to challenge 84 days following treatment. Callow and McGregor (1970) suggested that cattle sterilised of *Babesia* spp. infections by excessive IMDC treatment may suffer a clinical attack upon further exposure to babesiosis. These findings disagree with those of Hart *et al.* (1971) and Wood (1971), whose results indicate that IMDC did not interfere with the development of immunity to bovine babesiosis. Because the calves in our experiment had

demonstrable *Babesia* spp. parasitaemias for only 3 days before treatment, the duration of immunity may be related to the longevity of initial infection prior to chemotherapy.

The ascending *A. marginale* parasitaemias of acute infections were rapidly decreased by 2.0 and 2.5 mg/kg of IMDC but not by 1.0 mg/kg. The reduction in *A. marginale* parasitaemias was directly related to increasing dosages of IMDC. These findings closely agree with the observations of Hart *et al.* (1971) and Roby (1972). The occurrence of recrudescing and/or persisting *A. marginale* parasitaemias and the associated reduction in PCV was relatively augmented as the dosage of IMDC increased which agrees rather well with the results of Hart *et al.* (1971). Sterilisation of anaplasmosis did not occur following treatment because the *A. marginale* CF antibody titres and parasitaemias were persistently present and only a mild response resulted following homologous challenge. Kuttler (1972), demonstrated that three intramuscular injections of 24 hour intervals of 4.0, 5.0 or 6.0 mg/kg of IMDC eliminated the *A. marginale* carrier status of splenectomised calves. Roby and Mazzola (1972), showed that two intramuscular or subcutaneous injections at 14 day intervals of 5.0 mg/kg of IMDC or imidocarb dipropionate eliminated the *A. marginale* carrier status in intact adult cattle. Therefore, it is apparent that increased amounts of IMDC are required for complete elimination of bovine anaplasmosis.

One calf treated with 1.0 mg/kg of IMDC and two calves treated with 2.0 mg/kg of IMDC died of either subacute or chronic recrudescing anaplasmosis on days 10, 21 and 56 PT, indicating that these dosages were probably not adequate to control the relapsing infection. All of the treated groups of calves had higher average daily weight gains than the control calves, except Group II which had lower weight gains apparently related to the continuous recurrence of anaplasmosis.

The dual chemotherapeutic efficacy of IMDC against bovine anaplasmosis and babesiosis is very beneficial. However, smaller doses, which are adequately effective against babesiosis, appear to have a minimal effect on anaplasmosis. Conversely, larger doses which are adequately effective against anaplasmosis may be too effective against babesiosis and sterilise the infection and possibly make the cattle resusceptible to babesiosis upon the next exposure. The diversity of the chemotherapeutic efficacy of a single dose of IMDC against concurrent bovine anaplasmosis and babesiosis in relation to the sterilization and re-exposure to babesiosis, warrants further evaluation. In addition, the relationship between the dosage of IMDC and the occurrence and severity of recrudescing anaplasmosis requires further elucidation.

ACKNOWLEDGEMENTS

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EFFICACITÉ CHIMIOTHÉRAPEUTIQUE DE L'IMIDOCARB DIHYDROCHLORIDE DANS L'ÉLIMINATION DE L'ANAPLASMOSE BOVINE ET DE LA BABÉSIOSE SIMULTANÉES. I. LES EFFETS D'UNE UNIQUE TRAITEMENT

Résumé—L'efficacité de ce produit administré à l'occasion d'une unique injection à la dose de 1,0, 2,0 et 2,5 mg/kg contre l'anaplasnose et la babésiose simultanées est rapportée. La dose

de 2 à 2,5 mg/kg inhibe rapidement le développement aigu de parasitémies dues à la fois à *Anaplasma marginale*, *Babesia bigemina* et *Babesia argentina*; cependant la dose de 1 mg/kg n'a qu'un effet modeste sur *A. marginale*, alors qu'il conserve toute sa valeur contre *B. bigemina* et *B. argentina*.

A la dose de 1,0, 2,0 et 2,5 mg/kg, ce corps entrave le développement de l'immunité dans les accès aigus dus à *Babesia* spp. rendant les veaux plus sensibles à de nouvelles attaques de babésioses. L'inhibition de parasitémie à base de *A. marginale* est directement en rapport avec l'accroissement de la dose d'Imidocarb dihydrochloride; toutefois, la recrudescence et la persistance après traitement de parasitémies sont plus fréquentes après usage de doses élevées.

LA EFICIENCIA QUEMOTERAPEUTICA DEL IMIDOCARB DIHYDROCLORIDE POR ELIMINACION DE LA ANAPLASMOSIS Y BABESIOSIS BOVINOS CONCURRENTES. I. LOS EFECTOS DE TRATAMIENTO UNICO

Sumario—La eficiencia quemoterapéutica del imidocarb dihydrochloride (3,3'-bis (2-imidazolín-2 yl) carbanilide dihydro chloride) administrada como dosis intramuscular unica de 1.0, 2.0 y 2.5 mg/kg, en infecciones concurrentes de anaplasmosis y babesiosis es reportada en este trabajo. Dosis de 2.0 y 2.5 mg/kg de imidocarb dihydrochloride, rapidamente inhibieron las parasitemias concurrentes ascendientes y agudas de *Anaplasma marginale*, *Babesia bigemina* y *Babesia argentina*; sin embargo, 1.0 mg/kg tuvo un efecto minimo sobre *A. marginale*, pero fue muy efectivo contra *B. bigemina* y *B. argentina*. El imidocarb dihydrochloride en dosis de 1.0, 2.0 y 2.5 mg/kg inhibió el desarrollo de inmunidad contra la forma aguda de *Babesia*, haciendo a los terneros mas susceptibles a la babesiosis al desafio. La inhibición de parasitemias de *A. marginale* estuvo directamente relacionada al aumento de la dosis de imidocarb dihydrochloride; sin embargo, parasitemias debido al recrudescimiento ó a la persistencia despues del tratamiento, tambien ocurrieron mas frecuentemente en dosis altas.

THE CHEMOTHERAPEUTIC EFFICACY OF IMIDOCARB DIHYDROCHLORIDE ON CONCURRENT BOVINE ANAPLASMOSIS AND BABESIOSIS

II. The Effects of Multiple Treatments

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SUMMARY

Intact Anaplasma marginale, Babesia bigemina and Babesia argentina carrier calves treated intramuscularly five or ten times with 2.5 mg/kg of imidocarb dihydrochloride at 48 hour intervals, eliminated the Babesia infections but not Anaplasma infections. The parasitaemias became microscopically undemonstrable within 4 days following the first treatment and the packed cell volumes increased significantly within 18 days. Intoxications resulting in fatalities occurred in five of six calves given 10 intramuscular treatments of 2.5 mg/kg of imidocarb dihydrochloride at 48 hour intervals.

INTRODUCTION

Imidocarb dihydrochloride¹ (3,3'-bis(2-imidazolin-2-yl) carbanilide dihydrochloride) (IMDC) was shown to be highly effective in the treatment and control of bovine babesiosis by Callow and McGregor (1970), Brown and Berger (1970), Roy-Smith (1971) and Hart, Roy-Smith, Berger, Simpson and McHardy (1971). Callow and McGregor (1970), Berger quoted in Brown and Berger (1970) and Hart *et al.* (1971) showed that single doses of IMDC down to 0.6 mg/kg sterilised *Babesia bigemina* infections. Callow and McGregor (1970) further demonstrated that single doses down to 2 mg/kg sterilised *Babesia argentina* infections of cattle.

Hart *et al.* (1971) and Roby (1972), demonstrated the high degree of chemotherapeutic efficacy of IMDC in the treatment of bovine anaplasmosis. Kuttler (1971), reported that three intramuscular treatments at 24 hour intervals of IMDC at base weight 4, 5, or 6 mg/kg sterilised *Anaplasma marginale* infections in splenectomised carrier calves. Roby and Mazzola (1972) showed that two subcutaneous or intramuscular injections at 14 day intervals of IMDC or imidocarb dipropionate at salt weight 5.0 mg/kg eliminated the *A. marginale* infection in intact adult carrier cattle.

This paper presents the results of an attempt to sterilise concurrent *A. marginale*, *B. bigemina* and *B. argentina* infections of intact carrier cattle with imidocarb dihydrochloride.

MATERIALS AND METHODS

Twelve *A. marginale*, *B. bigemina* and *B. argentina* carrier, 430 ± 18 days old, male, intact Holstein-Friesian calves were used in the experiment which was executed in tick-free quarters at the Instituto Colombiano Agropecuario, Laboratorio de Investigaciones Medicas Veterinarias located in Bogotá, Colombia (altitude 2,600 m). Each of the 12 calves was previously inoculated subcutaneously on day -41 with 10 ml of blood containing 3×10^9 *A. marginale* bodies and day -38 with 10 ml of blood containing a mixture of 9×10^7 *B. bigemina* and *B. argentina*. The parasites were identified by the examination of Giemsa-stained thin blood films. The number of parasites was estimated by determining in quadruplicate the percentage

¹ Imidocarb, Burroughs-Wellcome & Co. Ltd., London, England.

of organisms in Giemsa-stained thin blood films and multiplying the average percentage of parasitaemia by the average total erythrocyte count per cubic centimetre, also determined in quadruplicate. The isolates of hemoparasites were obtained from a splenectomised calf exposed to a natural environment where *Boophilus microplus* were abundant in a pasture located at the Turipaná Instituto Colombiano Agropecuario experimental farm near Montería, Córdoba, Colombia (altitude 24 m). The isolates were further twice passaged by inoculating 100 ml of blood into intact calves to help eliminate the possibility of concurrent infectious diseases. The calves were demonstrated to be carriers by the presence of positive parasitaemias, positive titres of complement fixing (CF) antibodies for *A. marginale*, *B. bigemina* and *B. argentina* and decreasing packed cell volumes (PCV). The carrier-calves were in poor physical condition as evidenced by weight loss, roughened hair coats and generalised debilitation.

Imidocarb dihydrochloride salt was dissolved in sterilised, distilled water to make a 5 per cent w/v solution. The drug was injected five times intramuscularly on days 0, 2, 4, 6 and 8 at 2.5 mg/kg into the six calves of Group I. The six calves of Group II were injected 10 times intramuscularly on days 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18 with 2.5 mg/kg of IMDC.

Fifty millilitre aliquots of blood collected on day 81 from each of the six calves of Group I were pooled and inoculated intravenously into a splenectomised calf. A 100 ml aliquot of blood collected on day 81 from the one surviving calf of Group II was inoculated intravenously into another splenectomised calf. Blood samples from each of the two splenectomised calves were collected daily for 2 weeks, and thereafter, twice weekly for 60 days for the detection of parasitaemias.

The interval of collection of blood and serum samples of Groups I and II was as depicted in Figs. 1 and 2. Blood samples were collected from the jugular vein using 1.2 g/l of disodium ethylenediamine tetra-acetate as anticoagulant. The packed cell volumes (PCV) were determined by the microhematocrit method after spinning for 5 minutes at 11,500 rev/min in a microhematocrit centrifuge. Combination thin and thick Giemsa-stained blood films were processed according to a modified method of Mahoney and Saal (1961). Parasitaemias for *A. marginale* and *Babesia* spp. were ascertained by examining a transect (75 fields using a $\times 100$ oil immersion objective with $\times 10$ oculars) approximately 3 to 6 mm from the tail of the thin blood films.

All complement-fixation (CF) serum antibody titres were performed by the microtitre method described by Hidalgo and Dimopoulos (1967). *Anaplasma marginale* CF antibody titres were determined by a microprocedural adaptation of a standard method (Anonymous, 1958), using the standard United States Department of Agriculture, Agriculture Research Service antigen. *Babesia bigemina* and *B. argentina* CF antibody titres were ascertained by a microprocedural adaptation of the method described by Mahoney (1962), using distilled water suspensions and extracts of parasitised bovine erythrocytes as antigens according to the method of Mahoney (1967).

RESULTS

The results of the five and ten treatments of 2.5 mg/kg of IMDC of Groups I and II respectively are given in Figs. 1 and 2. Each point on the curves of the graphs represents the mean and the bar through each point indicates one standard deviation for a given day. From day 0 to day 18 the PCV of Groups I and II increased significantly ($P < 0.01$) from 23.5 ± 2.9 per cent to 34.8 ± 1.5 per cent and from 23.0 ± 4.0 per cent to 33.0 ± 2.0 per cent respectively. Following day 18 the PCV of both groups remained at approximately normal levels. By day 4, the *A. marginale*, *B. bigemina* and *B. argentina* parasitaemias became microscopically undemonstrable and remained so throughout the rest of the experiment.

Calves treated 10 times with IMDC developed negative CF titres for *A. marginale*, *B. bigemina* and *B. argentina* 10 to 20 days before calves treated five times, and all cattle had negative titres by day 60. The *Babesia* CF titres usually became negative before the *Anaplasma* titres.

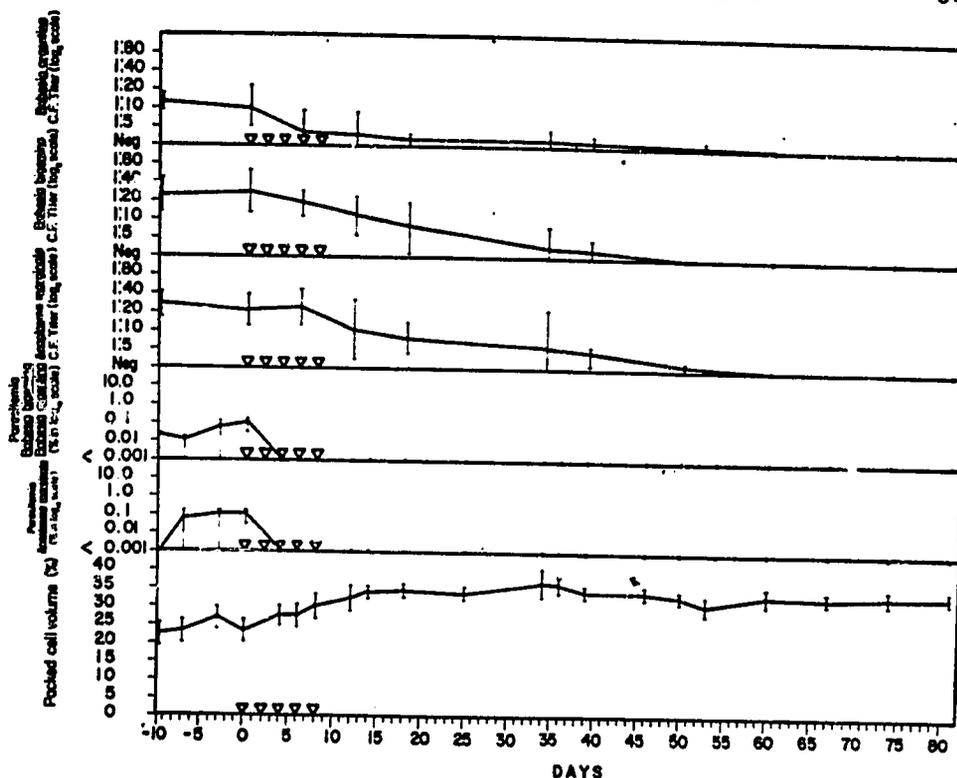


FIG. 1. Haematologic and serologic results of six concurrent *A. marginale*, *B. bigemina* and *B. argentina* carrier calves (Group I) given five intramuscular treatments of 2.5 mg/kg of imidocarb dihydrochloride. The mean and one standard deviation are represented for each given day. ▽ = day of treatment.

From day 0 to 10, Group I had an average daily weight loss of 36 ± 22 g/day and from day 0 to 18, Group II had an average daily weight loss of 112 ± 56 g/day. From day 10 to 81, Group I had an average daily gain of 358 ± 137 g/day, and from day 18 to 81, the surviving calf of Group II had an average daily gain of 196 g/day. The physical status of the calves of Group I improved markedly but the physical status of the calves of Group II remained static.

No mortalities occurred in Group I. However, five of six calves of Group II died on days 15, 18, 19, 36 and 63 with the following macroscopic lesions: petechiation of serosal surfaces, hydrothorax, hydroperitoneum and enlarged, pale, swollen kidneys. Excessive salivation, lachrymation and mild dyspnoea persisted for 15–30 minutes following each inoculation and repetitive treatments did not increase the severity or duration of the signs. Minimal local inflammatory responses occurred at the sites of injection but usually disappeared within 24 hours.

Neither of the splenectomised calves inoculated with pooled blood from Groups I and II demonstrated any evidence of *B. bigemina* or *B. argentina*. The splenectomised calf subinoculated with pooled blood from Group I developed an *A. marginale* parasitaemia on day 22 post-inoculation and died on day 34 with a PCV of 8.0 per cent and a parasitaemia of 16.0 per cent. The splenectomised calf inoculated with the blood of the surviving calf of Group II developed an *A. marginale* parasitaemia on day 32 post-inoculation which increased to a maximum parasitaemia of 12 per cent on day 40 and a minimum PCV of 14 per cent on day 43.

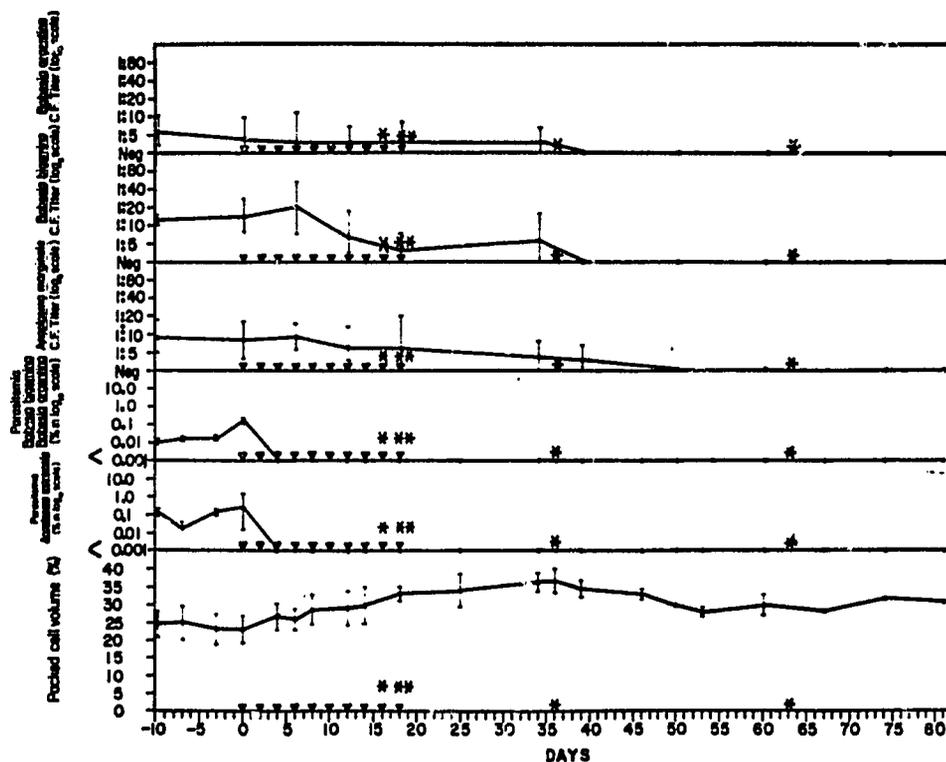


FIG. 2. Haematologic and serologic results of six concurrent *A. marginale*, *B. bigemina* and *B. argentina* carrier calves (Group II) given 10 intramuscular treatments of 2.5 mg/kg of imidocarb dihydrochloride. The mean and one standard deviation are represented for each given day. ▽ = day of treatment and * = death of one calf.

DISCUSSION

Imidocarb dihydrochloride rapidly inhibited the parasitaemias of *A. marginale*, *B. bigemina* and *B. argentina*, making them microscopically undemonstrable within 4 days following the first treatment of 2.5 mg/kg of IMDC. The inhibitory effect of IMDC on the parasitaemias was paralleled by rapidly increasing PCV and an improvement in the physical status of the calves following treatment.

The babesiosis carrier status of intact calves was eliminated by five and 10 intramuscular treatments of 2.5 mg/kg of IMDC at 48 hours intervals which agrees with results of Callow and McGregor (1970), Berger quoted in Brown and Berger (1970) and Hart *et al.* (1971), who demonstrated lower single doses sterilised *Babesia* infections.

The anaplasmosis carrier status of intact calves was not eliminated by either five or ten intramuscular treatments of 2.5 mg/kg of IMDC at 48 hour intervals. Yet Kuttler (1971) sterilised *A. marginale* infections in splenectomised calves with three intramuscular treatments at 24 hour intervals of IMDC at base weights of 4, 5 or 6 mg/kg and Roby and Mazzola (1972) eliminated *A. marginale* infections in intact adult cattle with two intramuscular treatments of salt weight 5.0 mg/kg of IMDC or imidocarb dipropionate. Apparently, a minimal chemotherapeutic blood or tissue level of IMDC must be reached and maintained for the elimination of *A. marginale*

carrier infections which is more easily attained with higher dosages at preferably shorter intervals. However, the increased possibility of drug toxicity must be considered with this regimen. Ten intramuscular applications of IMDC at 2.5 mg/kg at 48 hour intervals was fatal in five or six calves. The calves had slight daily weight losses during the period of chemotherapy but made rapid weight gains following treatment, indicating that repetitive chemotherapy with IMDC may reduce metabolic efficiency and result in decreased weight gains of calves during the treatment period.

Roby and Mazzola (1972) indicated that elimination of the carrier state of anaplasmosis in adult cows should be accompanied by loss of CF antibody titres. Kuttler (1971) found that IMDC treated splenectomised calves which failed to relapse within 62 days following treatment were consistently free of anaplasmosis as determined by infectivity trials. Although intact calves are not directly comparable with splenectomised calves or adult cows, the intact calves in our experiment treated five or 10 times with IMDC at 2.5 mg/kg did not develop relapsing *A. marginale* parasitaemias within 81 days post-treatment and had negative CF *A. marginale* antibody titres by 60 days post-treatment, yet remained patent carriers of anaplasmosis upon subinoculation into splenectomised calves. These results suggest that a negative anaplasmosis CF antibody titre and the lack of relapsing parasitaemia are good indicators but not sufficient to verify elimination of the anaplasmosis carrier state, making subinoculation into splenectomised calves necessary for confirmation.

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**EFFICACITÉ CHIMIOTHÉRAPEUTIQUE DE L'IMIDOCARB DIHYDROCHLORIDE
DANS L'ÉLIMINATION DE L'ANAPLASMOSE BOVINE ET DE LA BABÉSIOSE
SIMULTANÉES. II. LES EFFETS DES TRAITEMENTS MULTIPLES**

Résumé—Des veaux porteurs sains d'*Anaplasma marginale*, *Babesia bigemina* et *Babesia argentina*, traités à 5 ou 10 reprises, en intramusculaire avec 2,5 mg/kg de ce corps, à 48 heures d'intervalle, éliminent les Babésies mais non les Anaplasmes. Les parasitémies deviennent microscopiquement nuisibles dans les quatre jours qui suivent la première injection et avec accroissement significatif, dans les dix huit jours, des éléments cellulaires sanguins. Des accidents mortels ont été observés sur cinq à six veaux, à la suite de traitements comportant dix injections intramusculaires de 2,5 mg/kg d'Imidocarb dihydrochloride à 48 heures d'intervalle.

**LA EFICIENCIA QUEMOTERAPEUTICA DEL IMIDOCARB DIHYDROCLORIDE
POR ELIMINACIÓN DE LA ANAPLASMOSIS Y BABESIOSIS BOVINAS
CONCURRENTES. II. LOS EFECTOS DE TRATAMIENTOS MULTIPLES**

Sumario—Terneros enteros portadores de *Anaplasma marginale*, *Babesia bigemina* y *Babesia argentina* tratados intramuscularmente cinco ó diez veces con 2.5 mg/kg de imidocarb dihydrochloride con 48 horas de intervalo, eliminaron las infecciones con *Babesia* pero no las de *Anaplasma*. Las parasitemias se tornaron indemostrables microscópicamente dentro de los primeros cuatro días de iniciado el tratamiento, y los volúmenes de hematocrito incrementaron significativamente dentro de 18 días. Intoxicaciones que resultaron en pérdidas ocurrieron en cinco de seis terneros sometidos a diez tratamientos intramusculares de 2.5 mg/kg de imidocarb dihydrochloride con 48 horas de intervalo.