



CIAT

COLECCION HISTORICA

Series CE-12
July 1977

Workshop
on
Hemoparasites
(Anaplasmosis and Babesiosis)

Editor: E.A. Wells

17-22 March, 1975

Cali, Colombia

Centro Internacional de Agricultura Tropical

CIAT is a nonprofit organization devoted to the agricultural and economic development of the lowland tropics. The Government of Colombia provides support as host country for CIAT and furnishes a 522-hectare farm near Cali for CIAT's headquarters. Collaborative work with the Instituto Colombiano Agropecuario (ICA) is carried out on several of its experimental stations and similar work is done with national agricultural agencies in other Latin American countries. CIAT is financed by a number of donors represented in the Consultative Group for International Agricultural Research. During this year these donors were the United States Agency for International Development (USAID), the Rockefeller Foundation, the Ford Foundation, the W.K. Kellogg Foundation, the Canadian International Development Agency (CIDA), the International Bank for Reconstruction and Development (IBRD) through the International Development Association (IDA), the Inter-American Development Bank (IDB) and the governments of Australia, Belgium, the Federal Republic of Germany, the Netherlands, Switzerland and the United Kingdom. In addition, special project funds are supplied by various of the aforementioned entities plus the International Development Research Centre (IDRC) of Canada and the International Board for Plant Genetic Resources (IBGPR). Information and conclusions reported herein do not necessarily reflect the position of any of the aforementioned agencies, foundations or governments.

CIAT
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Series CE - 12
July, 1977

Workshop
on



Hemoparasites

(Anaplasmosis and Babesiosis) *Cali, 1977*

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CIAT
BIBLIOTECA

44173

E. A. Wells, Editor

Hemoparasites (Anaplasmosis and Babesiosis) (Cali, 1977)

6321

Centro Internacional de Agricultura Tropical, CIAT

Apartado Aéreo 67-13 Cali, Colombia, S. A.

Cables CINATROP

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Grateful acknowledgement

The Hemoparasite Workshop was made possible by the encouragement and financial assistance of the U.S. Agency for International Development (USAID).

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PROGRAM

Monday 17 March.

Registration and Introduction.

Section 1. The epidemiology of bovine anaplasmosis and bovine babesiosis.

Tuesday 18 March.

Section 2. The control of bovine babesiosis

- a. Diagnosis
- b. Immunization
- c. Control of vectors.

Wednesday 19 March.

Section 3. The control of bovine anaplasmosis

- a. Diagnosis
- b. Immunization
- c. Control of vectors.

Thursday 20 March.

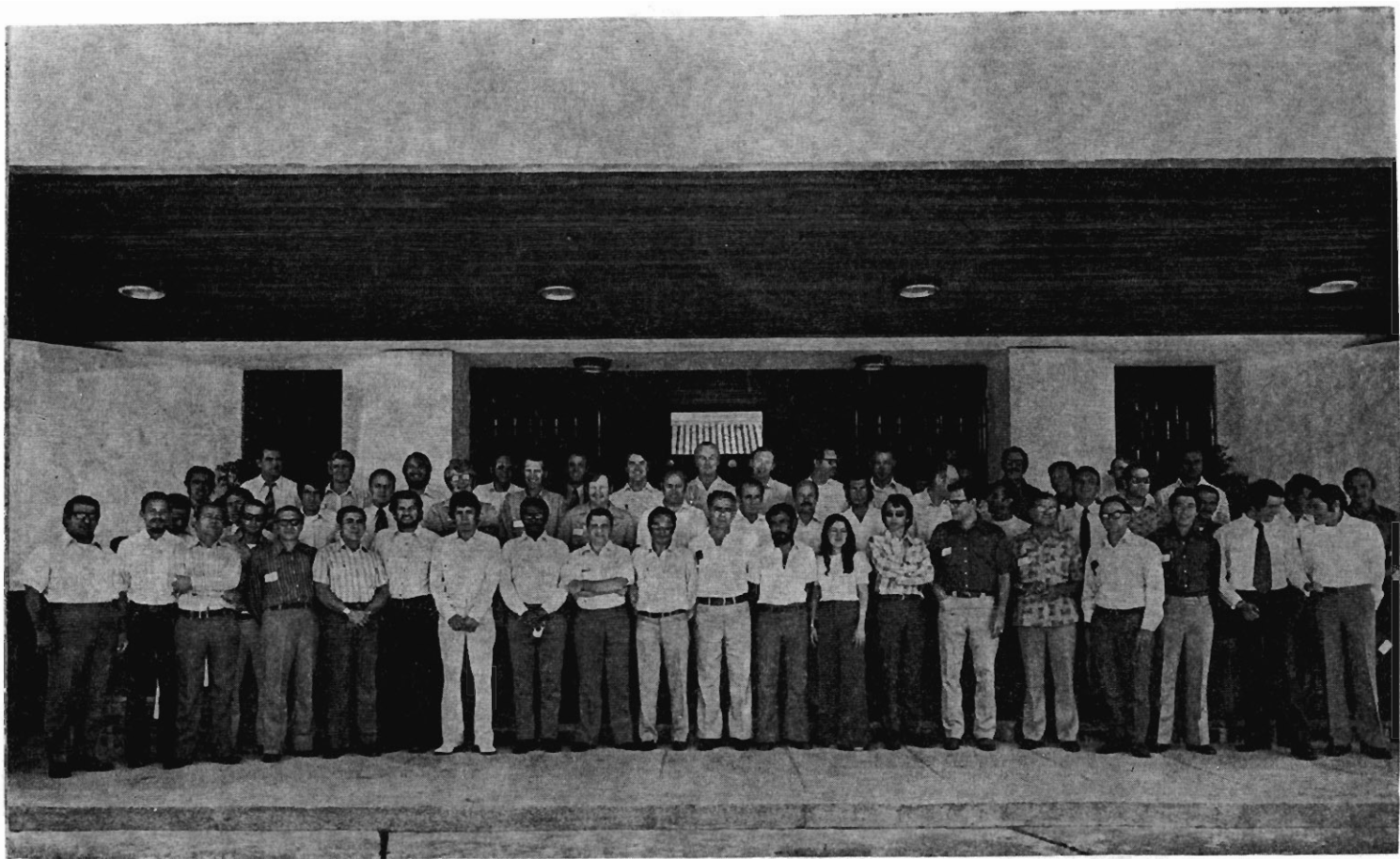
Field trip by bus to see working sites in the Cauca Valley, preceded by introductory explanation of the Texas A&M special project in hemoparasites carried out in collaboration with the Instituto Colombiano Agropecuario.

Friday 21 March.

Section 4. Research requirements for the control of bovine anaplasmosis and babesiosis in Latin America.

Saturday 22 March.

Field trip by air to the Carimagua station of the Instituto Colombiano Agropecuario in the Llanos Orientales to see the special problems of the region.



Participants in the Hemoparasite Workshop held at CIAT, Cali, Colombia (March 17-22, 1975).

INTRODUCTION

THE ANIMAL HEALTH PROGRAM IN CIAT

*E. A. Wells **

I want to speak for only about ten minutes. We have given you all a green booklet in Spanish or English in which we have given some information about our Animal Health group in CIAT. I merely want to enlarge on the information on the second page where we describe our animal health objectives.

We describe our program as consisting of:

a. Training in methodology through graduate programs; that is, how to relate an investigation laboratory to the field.

b. Research in problems of the greatest importance where present methods of prevention or control are inadequate.

c. Innovation in areas of present neglect; for example, animal health economics, animal health documentation and acarology.

I want to enlarge on these three areas.

We have evolved a simple methodology for the kind of applied work that we do. First, our laboratory acts as a team of disciplines. Our decisions are made on a team basis. The special projects that we have in hemoparasitology and in acarology are part of this integrated team approach. The second point is that we believe our research staff should move to the field where the problems are. The third point is that in the areas where we work we try to appreciate the total environment of the farm. The fourth point is that we hand over the results of our investigations to the extension veterinarians at the level of the farm. We do not meet him only at the door of the laboratory.

Our work in the Eastern plains of Colombia last year was on this pattern. A field team went out looking for a spectrum of information. The spectrum included breeding diseases (brucellosis, leptospirosis, IBR), hemoparasitic diseases (anaplasmosis, babesiosis), quantitative and qualitative work on ticks, quantitative work on *Dermatobia ho-*

* Leader, Animal Health Group.
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Apartado Aéreo 67-13, Cali, Colombia.

minis, questions on farm management and questions on farm economics. We, therefore, saw a dynamic picture of diseases in relation to each other and to their environment.

The first paper this afternoon will be a description by Dr. Corrier, of the Texas A&M group, of the results to date of the hemoparasite and tick portions of this work.

Unless this type of multidisciplinary team approach is used, we believe that the areas of required research may not be revealed, i.e. we may waste resources in the second area of our work. We may, for example, spend too much time on an immunizing procedure when farm management is revealed as a more crucial factor.

The third area of our work we describe as "innovations". They are innovations but they are also essentials. Let us mention only animal health economics. You will notice in our annual report that we have carried out some work on the impact of foot and mouth disease on pig farms. We are assembled here in this workshop because we all believe that anaplasmosis and babesiosis are important. However, can any of us put a figure in terms of money loss due to these diseases in Latin America? Are we attempting to assess the cost of different control strategies? The answers are simple. No, we cannot and we are not. Governments and donor agencies want to know where to put their money to greatest effect. We need to develop approaches to the economics of animal health.

Lastly, I want to mention graduate programs. If national agencies see any use in our activities, CIAT offers facilities for graduate training. Principally, this is in two forms. We offer facilities for M.S. and Ph.D. students to carry out their thesis research. We also take post graduate interns; that is, people who come for a maximum of one year to learn laboratory techniques and relate them to the field. In the area of animal health we are currently building up to ten students in total by mid year.

This workshop is to create an awareness of the problems in anaplasmosis and babesiosis in Latin America. If the workshop is a success we can offer our facilities for further workshops of more specific and detailed nature.

This is all the introduction I want to give. I sincerely hope that the contacts and friendships we are going to make this week will benefit the cattle industry of Latin America.

THE WORK OF THE INSTITUTE OF TROPICAL VETERINARY MEDICINE, COLLEGE OF VETERINARY MEDICINE, TEXAS A&M UNIVERSITY

*Fred Maurer **

The Institute of Tropical Veterinary Medicine was established in the College of Veterinary Medicine at Texas A&M University in 1966.

Texas provides an excellent site for such a program because of the subtropical climate, the very large domestic and wildlife populations and the proximity to Mexico, Central and South America.

The purpose:

To contribute to the more efficient control of tropical diseases of livestock through the conduct of research and the training of veterinarians.

Specific objectives are to:

1. Build a cadre of well-trained, experienced faculty;
2. Conduct research to improve the control and prevention of disease;
3. Establish a fellowship program for the training of graduate students in tropical veterinary medicine;
4. Maintain laboratories abroad for research and training in regional disease problems;
5. Collect and prepare a library of information and training aids for use in the program.

Support:

Financial and other support has been provided by:

The Rockefeller Foundation, 1967 - 1971;

* Director, Institute of Tropical Veterinary Medicine, Texas A&M University, College Station, Texas 77843, U.S.A.

U. S. Agency for International Development, 1968 to date;
Texas A&M has provided laboratory and animal facilities
on the campus at College Station, Texas;

The Rockefeller Foundation and the Colombian Government
thru ICA, provided laboratories and cooperative assistance
in Bogotá and Turipaná, Colombia;

Since 1971 we have worked as a part of the Animal Health program
here at CIAT.

Professional Staff:

We presently have 8 faculty members, all D.V.M., Ph.D.'s with
a total of over 30 years of experience in tropical countries. Those at
Texas A&M work in close cooperation with the total faculty of the
College of Veterinary Medicine and those at CIAT in cooperation
with CIAT personnel.

Post Graduate Training:

Since 1967, there have usually been 6 U.S. graduate students, with
3 in training in Texas, and 3 in Colombia.

These students do their academic work in Veterinary Microbiology,
Pathology or Parasitology in Texas, and the research for their
dissertations in Colombia.

Nine fellows have completed work for M.S. or Ph.D. degrees:
At present, 1 is pursuing a Ph.D.; 2 are seeking M.S. degrees.

In addition to these American graduate students in our fellowship
program, our staff has participated in the graduate training of several
foreign veterinarians both in Colombia and in Texas.

To facilitate our training program we are also engaged in the
accumulation of a library of information and training aids for the
major diseases of the tropics. And we will gladly talk with any of you
who may be interested in the exchange of training aids.

Research in Texas and Colombia:

Has emphasized the improvement of methods for the control of
hemoprotozoal diseases, especially anaplasmosis and babesiosis with
some work on theileriosis and trypanosomiasis.

Research Accomplishments:

Have been presented in our annual reports and in over 80

publications and some of this work will be detailed in the course of this meeting.

Briefly, these studies and accomplishments have included the following subject areas:

The incidence and distribution of anaplasmosis, babesiosis and trypanosomiasis in Colombia.

The card and CF tests for **Babesia** have been adapted for use with the Colombian strains.

The clinical, serological and pathological effects of single and concurrent infection with anaplasmosis and babesiosis have revealed dual infections to be additive.

The influence of external and gastro-intestinal parasites in animals infected with anaplasmosis and/or babesiosis has been recorded.

The antigenic character and pathogenicity of the hemoprotozoal organisms found in Colombia have been demonstrated.

Studies have confirmed that Colombian strains of **B. argentina** and **B. bigemina** are somewhat different immunologically. The **B. argentina** challenge of **B. bigemina** carriers produced more severe reactions than when the situation was reversed.

The resistance of these hemoprotozoal pathogens to known immunological procedures have been determined.

The vaccine currently available in the U.S. against anaplasmosis has been evaluated and found unsatisfactory in Colombia.

Vaccines prepared from killed, attenuated and fully virulent **Anaplasma marginale** have been tested and evaluated.

Premunition methods for the prevention of clinical infections with **Anaplasma marginale**, **Babesia argentina** and **Babesia bigemina** have been tried and improved.

Controlled premunition of cattle with **Anaplasma** and **Babesia** organisms of different known pathogenicity have been made and results compared.

Some of these trials have been very successful leading to more refined and extensive, controlled field trials which are now underway here in Colombia, and you will be given some of the details later in this meeting.

The pathogenicity of **Babesia** for premunition as influenced by irradiation and serial animal passage have been compared with fully pathogenic strains.

Premunition with or without drug therapy has been compared for both **Anaplasma** and **Babesia**.

Immunological studies have confirmed that a sterile immunity to **Babesia** can be induced in susceptible cattle in the absence of the carrier state.

Both tissue cultures and blood cell cultures are being maintained for the growth of **Anaplasma** and **Babesia**. To date, only **Anaplasma** have been grown successfully on tissue cultures.

The incidence, distribution and the cycle of infection between the tick vector and the host is being studied in relation to both **Anaplasma** and **Babesia**.

The feeding of **Babesia** infected ticks on white tailed deer failed to induce infection in the deer. The injection of **Babesia** infected bovine blood into deer also failed to infect.

The therapeutic, prophylactic and toxic affects of drugs are being evaluated for the treatment and control of hemoprotozoa.

Imidocarb has been demonstrated to have prolonged prophylactic value against Colombian and Mexican strains of **Babesia** and to prevent or eliminate infection in the tick vector.

We realize there is still much to be done to improve the control of these diseases. Premunition, while very helpful, (especially for the protection of clean cattle moving into infected areas) is a cumbersome method which needs adjustment to local situations.

Chemotherapy and chemoprophylaxis have a place and a good potential providing effective drugs such as Imidocarb and Ganaseg can be cleared as safe for use by involvement Governments.

Effective one-shot and forget-it vaccines are needed for each of these diseases, especially in regions where vector eradication is likely to be many years away.

The need for research on these diseases is currently intensified by the world-wide difficulty of providing adequate food for the human population.

As you are all aware, the world scarcity of food has been brought on by several things:

1. Primarily, by the uncontrolled growth of the human population;
2. The shift of rural people to the cities;
3. The increased usage of cereal grains with the improvement of diets in the developed countries;

4. Poor food storage;
5. The higher costs of fertilizers, pesticides, and oil;
6. Adverse weather conditions, and
7. The burden of animal disease.

While animal disease is only one of the handicaps to food production it is a vital one because of the necessity for animal protein in man's diet to provide vitamin B₁₂, phospholipids essential for C.N.S. development and for those amino acids which are generally inadequate in vegetarian diets.

Further, only ruminants can convert the vast (65%) rangeland forages of the world to food for man.

For these reasons we are enthusiastic about efforts to control these diseases and most grateful to all of you attending this workshop for your cooperative interest in these diseases.

It is also appreciated that efficient livestock production requires expertise in animal breeding, reproduction, nutrition and management so that we are grateful for the opportunity to work here with CIAT where research is conducted in all these areas.

We are also involved in a 4-University consortium in the States which represent these disciplines in their relation to livestock production in the tropics.

KEYNOTE PAPERS

THE EPIDEMIOLOGY OF BOVINE ANAPLASMOSIS AND BABESIOSIS IN THE LOWLAND TROPICS OF COLOMBIA

*Donald E. Corrier **

Introduction

The epidemiology of anaplasmosis and babesiosis in Colombia is influenced by several geographical factors. Colombia is divided longitudinally by 3 cordilleras of the Andean mountain range (2). The western cordillera separates the narrow Pacific coast lowlands from the interior of the country. The central cordillera divides Colombia's 2 major valleys formed by the Cauca and the Magdalena rivers; while the eastern cordillera separates the western part of the country from the low eastern plains, los Llanos Orientales, which comprises nearly three fifths of Colombia's surface area and extends nearly 640 kilometers eastward to the Venezuelan border (2).

Three major climatic zones exist, reflecting differences in elevation associated with the 3 cordilleras of the Andes (2). A hot, lowland zone is found below 800 meters with an average temperature of 25° C. An intermediate zone occurs at 800 to 2100 meters with a temperature range of 17 to 23° C, while a high zone occurs above 2100 meters with an average temperature of 13° C or lower.

The lowland tropics of Colombia are found below 1000 meters, have an average temperature range of approximately 24 to 28° C and are found along the Pacific coast; the Atlantic coast; within the Cauca and Magdalena river valleys; and in los Llanos Orientales (2).

Colombia's estimated 3 million dairy cattle are found in the high mountainous zone and in the intermediate climatic zone (12). Beef cattle number approximately 19 million head and are found in the hot lowlands and in the intermediate climatic zone (12).

Although it is generally known that anaplasmosis and babesiosis are widespread in Colombia, especially in the lowland tropical zone,

* Texas A&M Special Project in Hemoparasites, Centro Internacional de Agricultura Tropical (CIAT), Apartado Aéreo 67-13, Cali, Colombia.

little epidemiological information is available. In 1974, 21 of 24 central diagnostic laboratories of the Instituto Colombiano Agropecuario (ICA), reported cases of anaplasmosis, while 18 laboratories reported cases of bovine babesiosis (15).

Kuttler, Adams and Zaraza reported the results of an epidemiological study of anaplasmosis conducted on 5 ICA experimental stations in 1969 (8). The stations were situated in different climatic zones ranging in altitude from 13 to 2600 meters with mean temperatures of 28°C and 13°C respectively. A direct correlation was noted between mean temperature and the prevalence of anaplasmosis (8). They reported the prevalence of anaplasmosis as 0% at 2,600 meters; 51% at 1,200 meters; 63% at 1,000 meters; 68% at 450 meters; and 91% at 13 meters. Other information is available indicating the absence of anaplasmosis and babesiosis at elevations of 2,600 meters and higher (4).

Epidemiological information concerning the incidence and distribution of anaplasmosis and babesiosis; the delineation of endemic from nonendemic and marginal areas; and a knowledge of the population and distribution of vectors and potential vectors; are items of basic information necessary in selecting effective prevention and control programs for anaplasmosis and babesiosis.

Epidemiological Studies

Three areas were selected for epidemiological studies:

1. the Eastern plains (los Llanos Orientales);
2. the North coast; and
3. the Cauca valley.

The area in los Llanos and the North coast are located in the lowland tropical zone, while the Cauca valley area is in the lower intermediate climatic zone. The 3 areas differ topographically, ecologically and in the systems of cattle management employed.

Epidemiological Study in the Eastern Plains

Materials and Methods

The study area extends from the piedmont region of the eastern Andean cordillera eastward approximately 450 kilometers through the Departament of Meta and into the Comisaría of Vichada. The area consists of savannah grasslands and varies in elevation from 500 meters in the piedmont region to 200 meters in the savannahs of Vichada. The average annual temperature is approximately 26° C with rainfall decreasing from 3000 mm. in the piedmont to 1,200 mm. in

Vichada. Rainfall is equally distributed during the wet season from April to November with little or no rainfall during the dry season from December through March (11). Cattle operations on the savannahs are extensive in nature with open rangetype management or minimum management being common. Ranches of 5,000 to 10,000 hectares are not considered unusually large. Average stocking rates of 0.4 animal units per hectare were reported for the Department of Meta (12), while stocking rates of 0.1 to 0.05 head per hectare were reported for the Llanos region in general (11). Cattle production on the savannahs is oriented toward the production of 3 to 4 year old feeders which are moved into the piedmont region and grass fattened for 8 to 10 months prior to slaughter. Predominant cattle breeds are Zebu, native Criollo cattle of Spanish descent, and Zebu-Criollo crosses.

The study area lies at the northern end of a zone of colonization which was reported to continue along the eastern Andean piedmont from Bolivia to Colombia and "represents one of the most active frontiers of agricultural settlement in interior South America" (3).

The study area was subdivided into 5 study zones based on distance from the piedmont region, and on differences in cattle operations and the level of ranch management.

Thirty-seven ranches were visited during the study and a total of 3,034 cattle examined. The ranches were selected in cooperation with the Caja Agraria "Cattle Development Program"*. Visits to the ranches were coordinated with routine visits by Caja Agraria personnel thus assuring an introduction onto the ranches and the establishment of favorable working relationships with ranch owners, managers and employees.

Each of the ranches was visited during the dry season from June to November and a random group of cattle representing a minimum of 10 per cent of the total herd were sampled ensuring that animals less than 1 year, 1 to 2 years, and more than 2 years of age were included in the sample group. The age, sex and breed of each animal was noted, and serum samples collected. Ticks were collected from infested cattle for later classification and the level of tick infestation assessed using a modification of a previously reported method (7). Tick counts were made on either the right or the left half of the animal's body. All ticks greater than 5 mm. in diameter were counted on one-half of the animal's body, excluding the inside of the ear. An area of 5 cm. square, in which the maximum number of ticks were located, was selected on the neck and on the inside of the hind leg and all ticks counted. All ticks inside one ear were counted distinguishing

* "Programa Desarrollo Ganadero". Caja de Crédito Agrario, Industrial y Minero Ministerio de Agricultura.

between ticks greater than and less than 5 mm. in diameter. Ticks greater than 5 mm. in diameter were considered to be engorged females, while ticks less than 5 mm. in diameter were considered as larvae, nymphs and adult males. The sum total of all ticks counted was expressed as the mean number of ticks per animal per herd. The total number of ticks greater than 5 mm. in diameter from the half body and ear counts was expressed as the mean number of ticks greater than 5 mm. per animal per herd. The individual counts from each animal were multiplied by 2 prior to herd calculations to reduce the number of mean values expressed as fractions.

The serum samples were tested for anaplasmosis using the Complement-Fixation (CF) screen test (1). Serum samples were tested for babesiosis, due to infection with *Babesia bigemina*, using a previously described modification (14) of the original reported CF technique (9). Test results were read as negative, trace, or 1+, 2+, 3+ and 4+ reactions. All 1+, 2+, 3+ and 4+ reactions were considered as reactors.

A study questionnaire designed to obtain information on herd history, herd management, animal health practices and the occurrence of various cattle diseases was completed for each herd. The information obtained was used to determine if statistically significant relationships existed between the prevalence of anaplasmosis in the herd and a number of selected variables. The variables analyzed included: level of tick infestation; estimated deer population in the area of the ranch; ranch size; number of cattle on the ranch; pasture management to include continual and rotational grazing; addition of new cattle to the herd during the past year; animal health cost per herd to include cost of vaccines, drugs and salt; the number of vaccinations per animal per year, coded to include 5 different vaccinations; and numerous management factors used to assess management level, coded to include individual identification of cattle, presence and condition of corrals, presence and condition of fences, presence and condition of cattle chute, presence of salt and salt boxes, presence of a tractor and equipment, presence of an electric generator, number of acres of improved pasture, and the presence and condition of spray race or dipping tank for tick control. The mean prevalence of anaplasmosis and the mean number of ticks per animal in each of the 5 zones within the study area were also submitted to analysis to determine if the differences in anaplasmosis prevalence or in tick infestation between the zones was statistically significant.

Results

Seventy-five per cent of the 3034 cattle tested were *Anaplasma* reactors (Table 1). Reactors were present in each of the 37 herds

Table 1. Results of Complement-Fixation Test for anaplasmosis.

Epidemiological study in the Eastern plains of Colombia,
June-November 1974.

Herd No.	No. of Cattle in Herd	No. of Cattle Tested	No. of Reactors *	Percent Reactors
1	445	45	42	93%
2	232	113	81	72%
3	484	99	56	57%
4	168	100	57	57%
5	455	108	67	62%
6	380	52	44	85%
7	386	60	40	67%
8	866	74	40	54%
9	254	53	33	62%
10	432	64	33	52%
11	326	85	49	58%
12	1060	99	97	98%
13	227	94	91	97%
14	1022	110	103	94%
15	1309	100	86	86%
16	1170	98	69	70%
17	1480	99	91	92%
18	1006	94	78	83%
19	867	100	75	75%
20	532	60	56	92%
21	523	83	58	70%
22	125	49	48	98%
23	247	70	63	90%
24	1430	100	74	74%
25	1340	100	75	75%
26	153	79	72	91%
27	246	87	74	85%
28	244	70	57	81%
29	968	103	83	80%
30	980	100	92	92%
31	620	55	39	71%
32	654	60	37	62%
33	400	100	48	48%
34	293	43	24	56%
35	357	30	19	63%
36	400	100	49	49%
37	545	99	55	56%
Total	22,626	3034**	2262	75%***

* 1+, 2+, 3+ and 4+ reactions C.F. screen.

** Represents a sample size of 13% of the total number of cattle in the 37 herds.

*** Percent reactors in the 37 herds.

Table 2. Ages of anaplasmosis reactors.

Epidemiological study in the Eastern plains of Colombia,
June-November 1974.

Age (Months)	No. of Cattle tested	No. of Reactors *	Percent Reactors
1-3	166	81	49%
4-6	248	203	82%
7-12	454	365	80%
13-24	631	441	70%
< 24	1491	1125	76%
Total	2990	2215	75% **

* 1+, 2+, 3+, 4+ reactions C.F. screen test.

** Percent reactors in all cattle tested.

with 18 herds having 75 per cent or more reactors, and 19 herds with 48 to 74 per cent reactors. The percentage of *Anaplasma* reactors in the 37 herds ranged from 48 to 98 per cent.

Forty-nine per cent of the calves tested between 1 and 3 months of age were *Anaplasma* reactors while 82 per cent of the calves between 4 and 6 months were reactors (Table 2). Eighty per cent reactors occurred in the 7 to 12 month old group; 70 per cent in the 13 to 24 month group; and 76 per cent of all cattle greater than 24 months of reactors increasing as the number of vaccinations increase.

The differences in the prevalence of anaplasmosis between the 5 zones within the study area were not significant (Table 3). None of the variables selected for analysis proved significant in accounting for differences in the prevalence of anaplasmosis among the 37 herds. Though not apparently significant, a trend was noted which would suggest that *Anaplasma* reactors within a herd may be associated with the number of vaccinations per animal per year, with the number of reactors increasing as the number of vaccinations increase.

Forty-two per cent, 1270 of the 3,034 cattle tested, were reactors to *B. bigemina* infection (Table 4). *Babesia* reactors were present in each of the 37 herds, with 17 herds having 46 per cent or more reactors and 20 herds with 5 to 41 per cent reactors. The *Babesia* reactors in the 37 herds ranged from 5 to 94 per cent.

Table 3. Variables used in statistical analysis of anaplasmosis prevalence.

Epidemiological study in the Eastern plains of Colombia,
June-November 1974.

Variable	Type of analysis	
	Analysis of variance	Pearson Products Moment Correlations
1. Difference in Prevalence between zones within the study area		NS*
2. Level of tick infestation:		
(1) Total number of ticks per animals		NS
(2) Number of adult ticks per animal		NS
3. Estimated deer population in area of farm		NS
4. Farm size in acres		NS
5. Number of cattle on the farm		NS
6. Pasture management:		
(1) Continual		NS
(2) Rotational		NS
7. Addition of new cattle during past year		NS
8. Animal health cost per herd: vaccines, drugs, salt, etc. ..		NS
9. Number of vaccinations per animal per year (coded)		NS
(1) Aftosa		
(2) Blackleg & Hemorrhagic Septicemia		
(3) Brucellosis		
(4) Anthrax		
(5) Salmonelosis		
10. Management factors (coded)		NS
(1) Individual identification of cattle		
(2) Presence and condition of corrals.		
(3) Presence and condition of fences		
(4) Presence and condition of cattle shute		
(5) Presence of salt & salt boxes		
(6) Presence of tractor & equipment		
(7) Presence of electric generator		
(8) Number of acres of improved pasture		
(9) Presence and condition of spray race or bath for tick control.		

* Not significant.

Table 4. Results of Complement-Fixation test for Babesia bigemina.

Epidemiological study in the Eastern plains of Colombia,
June-November 1974.

Herd No.	No. of Cattle in Herd	No. of Cattle Tested	No. of Reactors *	Percent Reactors
1	445	45	21	47%
2	232	113	36	32%
3	484	99	51	52%
4	168	100	28	28%
5	455	108	20	19%
6	380	52	30	58%
7	386	60	14	23%
8	866	74	12	16%
9	254	53	26	49%
10	432	64	25	39%
11	326	85	41	48%
12	1060	99	56	57%
13	227	94	44	47%
14	1022	110	85	77%
15	1309	100	46	46%
16	1170	98	46	47%
17	1480	99	38	38%
18	1006	94	36	38%
19	867	100	23	23%
20	532	60	48	80%
21	523	83	33	40%
22	125	49	46	94%
23	247	70	54	77%
24	1430	100	35	35%
25	1340	100	31	31%
26	153	79	54	68%
27	246	87	36	41%
28	244	70	47	67%
29	968	103	60	58%
30	980	100	59	59%
31	620	55	19	35%
32	654	60	13	22%
33	400	100	24	24%
34	293	43	2	5%
35	357	30	4	13%
36	400	100	8	8%
37	545	99	19	19%
Total	22,626	3034	1270	42% **

* 1+, 2+, 3+ and 4+ reactions C.F. screen test.

** Percentage of reactors in the 3034 animals tested.

Table 5. Ages of *Babesia bigemina* reactors.

Epidemiological study in the Eastern plains of Colombia,
June-November 1974.

Age (Months)	No. of Cattle tested	No. of Reactors *	Percent Reactors
1-3	144	66	46%
4-6	254	165	65%
7-12	416	269	65%
13-24	412	197	48%
> 24	660	194	30%
Total	1886	891	47%**

* 1+, 2+, 3+ and 4+ reactions C.F. screen test.

** Percent reactors in the 1886 cattle tested.

Forty-six per cent of the calves between 1 and 3 months of age were *Babesia* reactors, while 65 per cent of the calves between 4 and 6 months were reactors (Table 5). Sixty-five per cent of the 7 to 12 month old group were reactors; 48 per cent of the 13 to 24 months group; and 30 per cent of all cattle tested over 24 months of age were *Babesia* reactors.

Ticks collected from cattle during the study included: *Boophilus microplus*; *Amblyomma cajennense*; *Amblyomma triste* and *Anocentor nitens* (Table 6). *Boophilus microplus* was the only species found on

Table 6. Tick counts and tick species identified.

Epidemiological study in the Eastern plains of Colombia,
June-November 1974.

No. of herds in study	37
No. of cattle examined	3034
Mean No. all ticks/animal	30 (2-82)
Mean No. ticks >5mm/animal	4 (0-24)
Tick species identified*	No. herds where found
<i>Boophilus microplus</i>	37
<i>Amblyomma cajennense</i>	3
<i>Amblyomma triste</i>	3
<i>Anocentor nitens</i>	3

* Ticks identified by Dr. K. C. Thompson, D.V.M., Ph.D Acarology section Animal Health, C.I.A.T.

all the 37 ranches, while the other species were each found on 3 ranches. The differences in the number of ticks between the 5 zones within the study area was not significant. Differences in the level of tick infestation between individual ranches was related to the type of tick control program employed, and the period of time elapsed since the cattle were last sprayed or dipped.

Seven ranch managers reported cases of anaplasmosis in the past year. Cases of babesiosis were not reported. A disease complex, commonly referred to as "Secadera" was reported most often as the most important disease problem on the ranch. "Secadera", occurs most commonly during the dry season and is associated with a complex of probable causes of which nutritional stress, anaplasmosis and babesiosis are considered principal causes.

Epidemiological Study on the North Coast

Materials and Methods

The epidemiological study on the North coast was carried out on 4 ranches. Three of the ranches are located in the Department of Cordoba in the municipalities of Las Cordobas and Pueblo Nuevo, while the fourth ranch is located in the Department of Sucre in the municipality of Sampués. The ranches are located on the broad undulating Atlántic coastal plain which varies in elevation from sea level to a few hundred meters. The climate is tropical with an average annual temperature of 28° C (2).

Rainfall is equally distributed during the wet season from April to November with the dry season lasting from December through March. Cattle production is more intensive than that in the Llanos of Colombia with an average stocking rate of 1.9 animal units per hectare (12). Ranches of 300 to 600 hectares are average in size. Predominant cattle breeds in the area are Zebu, Native Criollo, and various Zebu crosses with Criollo, Holstein and other breeds.

The Departments of Cordoba and Sucre contain approximately 3 million and 1-1/2 million head respectively of Colombia's 19 million beef cattle (12) and are therefore 2 of the largest beef producing Departments in Colombia.

Thirty pregnant cows, in their sixth to ninth month of gestation, were selected on each of the 4 ranches and identified by ear tag, brand or tattoo number. A serum sample was collected from each cow prior to parturition and again within 2 weeks following parturition.

Abortions, death of several calves and infection with other diseases resulted in 112 calves being included in determining the age at first infection with anaplasmosis, and 107 calves being included in determining age at first infection with babesiosis.

Blood and serum samples were collected from each calf as soon after birth as possible and at biweekly intervals thereafter until the calves had reached 6 months of age. Giemsa stained thin blood films were prepared and examined microscopically to determine the earliest age at which parasitized erythrocytes could be detected and to determine the level of parasitemia in subsequent weeks. Mean parasitemias of **Anaplasma** and **Babesia** parasites were calculated so that week "O" corresponded to the week when infection was first diagnosed in each calf and subsequent levels of parasitemia corresponded to the same stage of infection in each calf, thus allowing maximum mean parasitemias to be observed.

Packed cell volumes (PCV) were determined using the micro-hematocrit technique (13). Changes in the mean PCV were calculated such that week "O" and subsequent weeks corresponded to the same stage of infection in each calf thus allowing maximum decreases in the PCV to be observed.

Serum samples collected from the cows and calves were tested for anaplasmosis using the complement-fixation (CF) test (1). The serum samples were tested for the presence of babesiosis using a previously described modification (14) of the original CF technique (9). Test results were read as negative, trace, or 1+, 2+, 3+ or 4+ reactions. All 1+, 2+, 3+ and 4+ reactions were considered reactors.

The age at which the calves were first infested by **Anaplasma marginale** and by **Babesia bigemina** was determined using the serological results and the results of blood smear examinations. Positive CF reactions which occurred in calves at 2 weeks of age were attributed to the presence of maternal antibodies and were not considered to be due to **Anaplasma** or **Babesia** infection.

The effect of first infections with **Anaplasma** and **Babesia** parasites was evaluated from the number of infected erythrocytes which were observed, and from the decreases which occurred in the PCV's.

Results

Ninety-nine of the 120 pregnant cows tested for anaplasmosis were reactors (Table 7). The reactors on the four ranches ranged from 63 to 100 per cent, with an overall prevalence of 83 per cent.

The mean age of infection with **Anaplasma** for the 112 calves included in the study was 11 weeks (Table 8). The earliest age of infection was 4 weeks with the latest age of infection being 24 weeks.

The mean parasitemias observed following infection with **Anaplasma marginale** were less than 1 per cent during the first week following

Table 7. Results of Complement Fixation test for anaplasmosis in pregnant cows.

Epidemiological study on the North coast of Colombia, November 1973-July 1974.

Name of Farm	No. of Cows Examined	No. of C.F. Reactors*	Percent C.F. Reactors
Las Delicias	30	23	77%
Sabana Acosta	30	30	100%
La Rebeca	30	19	63%
Nueva Colombia	30	27	90%
Total	120	99	83%**

* 1+, 2+, 3+ and 4+ reactions C.F. Screen Test.

** Percentage reactors in the 120 cows tested.

infection and decreased thereafter to less than 1 parasitized erythrocyte per 50 microscopic fields (Table 9).

Sixty-eight of the 120 pregnant cows tested for babesiosis due to infection with *B. bigemina* were reactors (Table 10). The reactors on the 4 ranches ranged from 17 to 80 per cent, with an overall prevalence of infection of 57 per cent.

Table 8. Age at first infection with *Anaplasma marginale*.

Epidemiological study on the North coast of Colombia, November 1973-July 1974.

Name of Farm	Number of Calves Examined	A at First Infection (weeks)	
		Mean	(Range)
Las Delicias	30	11	(6-24)
Sabana Acosta	26	12	(6-18)
La Rebeca	29	12	(4-24)
Nueva Colombia	27	9	(4-16)
Total	112*	11**	

* All of the calves (112) were infected by 24 weeks of age.

** Mean age of infection for all 112 calves.

Table 9. Parasitemia in calves following first infection with *Anaplasma marginale*

Epidemiological study on the North coast of Colombia,

November 1973-July 1974.

Stage of Infection (weeks)	Las Delicias Parasitemia (%)		Sabana Acosta Parasitemia (%)		La Rebeca Parasitemia (%)		Nueva Colombia Parasitemia (%)	
	Mean	(Range)	Mean	(Range)	Mean	(Range)	Mean	(Range)
	**							
0*	0.36	(<0.01-2.90)	0.88	(<0.01-6.00)	0.54	(<0.01-2.80)	0.82	(<0.01-5.80)
+2	0.32	(<0.01-2.10)	0.38	(<0.01-2.80)	0.17	(<0.01-1.40)	0.13	(<0.01-0.70)
+4	0.03	(<0.01-0.40)	0.02	(<0.01-0.15)	0.07	(<0.01-0.70)	0.06	(<0.01-0.90)
+6	0.06	(<0.01-0.70)	0.08	(<0.01-1.20)	<0.01	(<0.01-0.02)	0.10	(<0.01-1.60)
+8	<0.01	(<0.01-0.05)	0.18	(<0.01-0.10)	<0.01	(<0.01-0.07)	0.04	(<0.01-0.60)
+10	<0.01	(<0.01-0.03)	0.03	(<0.01-0.10)	0.11	(<0.01-1.00)	<0.01	(<0.01-0.01)
+12	<0.01	(<0.01-0.05)	0.01	(<0.01-0.10)	<0.01	(<0.01-0.01)	0.01	(<0.01-0.13)
+14	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.02)	<0.01	(<0.01-0.02)	<0.01	(<0.01-0.02)
+16	<0.01	(<0.01-0.05)	<0.01	(<0.01-0.03)	0.04	(<0.01-0.13)	<0.01	(<0.01-0.04)
+18	<0.01	(<0.01-0.03)	<0.01	(<0.01-0.03)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)
+20	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.03)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)

* Week "0" corresponds to the week when infection with *A. marginale* was first diagnosed in each calf.

** A parasitemia of <0.01 indicated that parasitized erythrocytes were not observed in approximately 10,000 cells or 50 microscopic fields.

Table 10. Results of Complement-Fixation tests for Babesia bigemina in pregnant cows.

Epidemiological study on the North coast of Colombia, November 1973-July 1974.

Name of Farm	No. of Cows Examined	No. of C. F. Reactors*	Percent C. F. Reactors
Las Delicias	30	5	17%
Sabana Acosta	30	18	60%
La Rebeca	30	21	70%
Nueva Colombia	30	24	80%
Total	120	68	57%**

* 1+, 2+, 3+ and 4+ reactions C.F. Screen Test.

** Percentage reactors in the 120 cows tested.

The mean age of infection with **B. bigemina** for the 107 calves included in the study was 11 weeks (Table 11). The earliest age of infection with **B. bigemina** was 2 weeks with the latest age of infection being 34 weeks.

The mean parasitemias observed following infection with **B. bigemina** seldom exceeded more than one infected erythrocyte per 50 microscopic fields (Table 12).

Table 11. Age at first infection with Babesia bigemina in pregnant cows.

Epidemiological study on the North coast of Colombia, November 1973-July 1974.

Name of Farm	Number of Calves Examined	Age at First Infection (weeks)	
		Mean	(Range)
Las Delicias	27	15	(4-26)
Sabana Acosta	25	13	(4-34)
La Rebeca	27	8	(2-22)
Nueva Colombia	28	7	(2-12)
Total	107*	11**	

* All of the calves (107) were infected by 34 weeks of age.

** Mean age of infection for all 107 calves.

Table 12. Parasitemia in calves following first infection with *Babesia bigemina*.

Epidemiological study on the North coast of Colombia,

November 1973-July 1974.

Stage of Infection (weeks)	Las Delicias Parasitemia (%)		Sabana Acosta Parasitemia (%)		La Rebeca Parasitemia (%)		Nueva Colombia Parasitemia (%)	
	Mean	(Range)	Mean	(Range)	Mean	(Range)	Mean	(Range)
0*	<0.01	(<0.01-0.02)	<0.01	(<0.01-0.03)	<0.01	(<0.01-0.03)	<0.01	(<0.10-0.04)
+2	<0.01	(<0.01-0.02)	<0.01	(<0.01-0.02)	<0.01	(<0.01-0.02)	<0.01	(<0.01-0.01)
+4	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.10-0.02)
+6	<0.01	(<0.01-0.02)	<0.01	(<0.01-0.02)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)
+8	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.02)	<0.01	(<0.01-0.03)
+10	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)
+12	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)
+14	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)
+16	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)
+18	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)
+20	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)	<0.01	(<0.01-0.01)

* Week "0" corresponds to the week when infection with *B. bigemina* was first diagnosed in each calf.

** A parasitemia of <0.01 indicated that parasitized erythrocytes were not observed in approximately 10,000 cells or 50 microscopic fields.

Decreases in the mean PCV's of the calves were not observed when calculated according to the age of the calves (Table 13). However, minimal decreases in the mean PCV's were observed when calculated according to the stage of infection with anaplasmosis (Table 14). The decreases in the mean PCV's occurred during the week when anaplasmosis was first diagnosed in each of the calves and during the second week following infection. The decreases observed were minimal with mean values not decreasing below 30 per cent. The low range limits observed in the PCV were 11, 14, 21 and 22 per cent.

Apparent clinical infection attributable to infection with anaplasmosis and/or babesiosis was observed in 2 calves. The calves appeared weak and listless and had PCV's of 11 and 14 per cent respectively. The calves were not treated for anaplasmosis or babesiosis and made an uneventful recovery with the PCV's returning to 30 per cent or more, 4 weeks following the signs of clinical illness.

Epidemiological Study in the Cauca Valley

Materials and Methods

The Cauca valley follows the Cauca river for approximately 250 kilometers extending north and south through the Department of Valle (2). The valley is located in the lower intermediate climatic zone and varies in elevation from approximately 900 meters on the valley floor to 1500 meters in the foothill regions. Rainfall is nearly equally distributed throughout the year, with the months of December and January receiving less rainfall than the remaining months of the year. Dairy cattle number slightly more than beef cattle in the Department of Valle with approximately 850,000 head of dairy cattle and 600,000 head of beef cattle reported (12). Dairy herds are found both in the intermediate climatic zone of the valley and foothills and also in the cool climatic zone in the mountainous regions of the cordilleras on either side of the valley. The movement of dairy cattle from the high mountainous zone down to the valley and foothills is considered to be dangerous by dairymen with losses considered to be due to anaplasmosis and/or babesiosis. Several stories have related losses to be as high as 50 per cent. Consequently dairy cattle are moved from the mountainous region down to the valleys only when destined for slaughter. A common management practice among the dairies is the confinement of calves until 6 to 10 months of age when they are first turned out to pasture. Clinical anaplasmosis and babesiosis was related to be frequently observed in the calves during the first few months on pasture.

Six dairy herds ranging in elevation from 930 to 1100 meters were visited during the study. Two of the herds were located in the southern end of the valley, 3 in the middle one-third, and one herd at the northern end of the valley. Information on herd history,

Table 13. Packed Cell Volumes in calves during first infection with *Anaplasma marginale* and *Babesia bigemina*.

Epidemiological study on the North coast of Colombia,
November 1973-July 1974.

Age of Calves (Weeks)	Las Delicias		Sabana Acosta		La Rebeca		Nueva Colombia	
	PCV (%)	(Range)	PCV (%)	(Range)	PCV (%)	(Range)	PCV (%)	(Range)
2	39	(34-45)	37	(31-42)	38	(30-48)	40	(37-45)
4	40	(32-48)	39	(30-43)	40	(30-48)	38	(32-43)
6	41	(26-48)	39	(12-46)	42	(33-48)	38	(35-43)
8	38	(22-46)	37	(11-47)	39	(21-46)	35	(14-46)
10	39	(28-52)	34	(19-45)	40	(29-47)	35	(23-51)
12	39	(28-51)	35	(19-46)	40	(26-48)	34	(15-51)
14	38	(24-48)	36	(25-48)	41	(34-48)	37	(30-49)
16	42	(32-51)	35	(22-47)	41	(35-46)	38	(27-46)
18	41	(28-48)	34	(28-46)	40	(30-47)	39	(31-46)
20	40	(33-51)	36	(29-46)	40	(33-45)	38	(29-42)
22	42	(32-49)	35	(28-43)	41	(31-50)	39	(32-49)
24	39	(33-51)	34	(25-42)	41	(31-51)	40	(35-50)
26	42	(35-47)	34	(24-38)	40	(35-45)	38	(32-48)
28	42	(35-50)	35	(28-40)	40	(36-47)	38	(31-44)
30	42	(32-50)	35	(32-40)	42	(36-45)	38	(34-43)
32	41	(37-45)	36	(32-40)	38	(31-45)	39	(35-43)
34	40	(33-46)	35	(30-39)	-*	(— —)	37	(35-40)
36	41	(36-46)	37	(34-42)	-	(— —)	38	(35-41)

* Information was not available.

Table 14. Packed Cell Volumes in calves during first infection with *Anaplasma marginale* and *Babesia bigemina*.*

Epidemiological study on the North coast of Colombia,
November 1973-July 1974.

Age of Infection (Weeks)	Las Delicias PCV (%)		Sabana Acosta PCV (%)		La Rebeca PCV (%)		Nueva Colombia PCV (%)	
	Mean	(Range)	Mean	(Range)	Mean	(Range)	Mean	(Range)
-8	40	(34-48)	40	(32-44)	42	(33-46)	—	(— —)
-6	41	(35-47)	40	(33-46)	42	(34-46)	41	(37-45)
-4	42	(34-46)	39	(33-46)	42	(33-47)	39	(32-46)
-2	42	(32-48)	39	(32-47)	42	(36-48)	41	(37-46)
0*	34	(22-44)	30	(11-40)	36	(21-44)	31	(14-45)
+2	37	(27-52)	33	(22-48)	38	(26-48)	35	(23-51)
+4	41	(28-51)	35	(22-48)	41	(33-50)	35	(25-51)
+6	41	(32-51)	36	(28-47)	42	(36-51)	38	(28-49)
+8	42	(33-51)	35	(23-46)	42	(36-48)	38	(30-46)
+10	42	(32-49)	36	(24-50)	40	(30-47)	38	(26-46)
+12	40	(33-50)	34	(25-41)	41	(37-45)	38	(32-45)
+14	41	(32-49)	34	(30-39)	41	(37-46)	40	(33-49)
+16	42	(33-49)	33	(29-39)	40	(31-45)	40	(31-50)
+18	41	(35-45)	36	(33-42)	39	(36-43)	39	(31-48)
+20	41	(32-50)	35	(30-40)	39	(36-43)	37	(32-43)

* Week "0" corresponds to the week when infection with *Anaplasma marginale* was first diagnosed in each calf.

animal health precautions and management practices was obtained on each herd. Serum samples were collected from a minimum of 10 per cent of the animals in the herd when possible.

The serum samples were tested for anaplasmosis and for babesiosis due to *B. bigemina* using the complement fixation test as described for the preceding studies. Test results were read as reported for the preceding studies.

Results

The results of the CF tests for anaplasmosis indicated there were 305 reactors which represented 71 per cent of the 432 cattle tested (Table 15). The *Anaplasma* reactors on the 6 ranches ranged from 60 to 96 per cent.

Twenty-three per cent of the calves tested between 1 and 6 months of age were *Anaplasma* reactors, while 63 per cent of the calves between 7 and 12 months were reactors (Table 16). Seventy-three per cent of the 13 to 24 month old age group were reactors and 83 per cent of all cattle greater than 24 months of age were *Anaplasma* reactors.

The results of the CF tests for *B. bigemina* indicated there were 319 reactors which represented 75 per cent of the 428 cattle tested (Table 17). The *B. bigemina* reactors on the 6 ranches ranged from 59 to 100 per cent.

Table 15. Results of Complement-Fixation tests for anaplasmosis.

Epidemiological study in the Cauca valley of Colombia,
March 1973-February 1974.

Herd No.	No. of Cattle in Herd	No. of Cattle Tested	No. of Reactors*	Percent Reactors
1	383	88	79	90%
2	300	23	22	96%
3	186	28	20	71%
4	196	32	23	72%
5	300	30	22	73%
6	243	231	139	60%
Total	1608	432	305	71%**

* 1+, 2+, 3+ and 4+ reactions C.F. screen test.

** Percentage of reactors in the 432 cattle tested.

Table 16. Ages of Anaplasma reactors.

Epidemiological study in the Cauca valley of Colombia,
March 1973-February 1974.

Age (months)	No. of Cattle Tested	No. of Reactors*	Percent Reactors
1-6	60	14	23%
7-12	64	40	63%
13-24	70	51	73%
> 24	146	121	83%
Total	340	226	67% **

* 1+, 2+, 3+ and 4+ Complement Fixation screen test.

** Percent reactors in the 340 cattle tested.

Twenty-seven per cent of the calves tested between 1 and 6 months of age were *B. bigemina* reactors, while 58 per cent of the calves between 7 and 12 months were reactors (Table 18). Seventy per cent of the cattle tested in the 13 to 24 month old age group were reactors and 94 per cent of all cattle tested greater than 24 months of age were *B. bigemina* reactors.

Table 17. Results of Complement-Fixation test for Babesia bigemina.

Epidemiological study in the Cauca valley of Colombia,
March 1973-February 1974.

Herd No.	No. of Cattle in Herd	No. of Cattle Tested	No. of Reactors*	Percent Reactors
1	383	88	81	92%
2	300	23	23	100%
3	186	28	25	89%
4	196	31	29	94%
5	300	30	27	90%
6	243	228	134	59%
Total	1608	428	319	75% **

* 1+, 2+, 3+ and 4+ reactions C.F. screen test.

** Percentage reactors in the 428 cattle tested.

Table 18. **Ages of Babesia bigemina reactors.**

Epidemiological study in the Cauca valley of Colombia,
March 1973-February 1975.

Age (months)	No. of Cattle Tested	No. of Reactors*	Percent Reactors
1-6	60	16	27%
7-12	50	29	58%
13-24	77	54	70%
> 24	139	131	94%
Total	326	230	71%**

* 1+, 2+, 3+ and 4+ Complement Fixation screen test.

** Percent reactors in the 326 cattle tested

DISCUSSION

Llanos study

The 75 per cent prevalence of *Anaplasma* reactors; the nonsignificant difference in the number of reactors between the 5 zones in the Llanos study area; and the presence of 82 per cent reactors in the 4

Table 19. **Epidemiological study of bovine anaplasmosis and babesiosis in the lowland tropics of Colombia.**

November 1973-February 1975.

Study Area	No. of Cattle Tested	Percent Reactors* <i>Anaplasma marginale</i>	Percent Reactors* <i>Babesia bigemina</i>
Eastern plains	3034	75%	42%
North coast	232	91%	77%
Cauca valley	432	71%	75%
Total	3698	75%**	48%***

* 1+, 2+, 3+, 4+ reactions Complement-Fixation screen test.

** Percentage anaplasmosis reactors in the 3698 cattle tested.

*** Percentage *Babesia bigemina* reactors in the 3698 cattle tested.

to 6 month old age group indicate that anaplasmosis is endemic and nearly equally distributed across the area of the Llanos surveyed. The high percentage of **Anaplasma** reactors in young calves, 49 per cent in the 3 to 6 month old group and 82 per cent in the 4 to 6 month old group, suggests that the majority of the calves are infected at an early age when maternal antibodies and natural resistance help to reduce the severity of the infection.

The decrease in the reactors from 82 per cent in the 4 to 6 month old age group to 76 per cent in the over 24 month age group would suggest that exposure to infection is less frequent in the older age group or that some of the older cattle were subclinical carriers and were not detected as reactors. The low number of ranches which reported clinical cases of anaplasmosis during the previous year further indicated that the majority of the cattle are infected at a young age when resistance to clinical disease is maximum and that subsequent re-exposure occurs at such frequency that few animals become fully susceptible to clinical anaplasmosis.

The uniformly high prevalence of **Anaplasma** reactors may have served to obscure the relationship of anaplasmosis prevalence to the variables included in the statistical analysis. The near significant relationship between prevalence and the number of vaccinations per animal per year appears to warrant further consideration. The practice of vaccinating all cattle in the herd for aftosa every 4 to 6 months, as is commonly done in the Llanos and in most of Colombia, may serve as an effective means of transmitting hemoparasitic diseases.

The 42 per cent prevalence of **B. bigemina** reactors in the Llanos indicates that although a large percentage of the cattle are infected 58 per cent remain susceptible to clinical babesiosis. The data would suggest an unstable situation in which clinical cases of babesiosis could be expected to occur in indigenous cattle. However, clinical cases of babesiosis during the previous year were not reported by any of the ranch managers on the 37 ranches. The large decrease in **Babesia** reactors from 65 per cent in the 7 to 12 month age group to 30 per cent in the age group over 24 months suggest that either a large proportion of the cattle are not reinfested following the initial infection, or that a large percentage of older cattle are subclinical carriers which were not detected by the CF test. The CF test may be expected to detect infection with **B. bigemina** for perhaps no longer than 4 months following a single infection (10). In addition, positive transmission tests have indicated that the number of subclinical cases of babesiosis may be higher than indicated by the CF test (5). The 30 per cent prevalence of **B. bigemina** in cattle over 24 months of age in

the Llanos, as detected by the CF test, was considered to be lower than the true number of cattle infected, many of which were probably subclinical carriers.

The 5 to 94 per cent variation in the number of *Babesia* reactors among the 37 herds in the Llanos study indicated the potential occurrence of clinical babesiosis associated with the movement of cattle from a herd with low prevalence to a herd with high prevalence.

The financial loss which anaplasmosis and babesiosis cause to cattle production in the Llanos is difficult to assess as reliable data on morbidity and mortality are not available and assessment must be based on information obtained from ranch owners and ranch managers, and is therefore dependent on the ability of the manager to differentiate anaplasmosis and babesiosis from other diseases. In addition, the range type management employed often results in acutely sick cattle dying before being observed. The prevalence of anaplasmosis and babesiosis in the Llanos area indicates that clinical cases of disease may occur in older cattle which were not exposed at a young age, or in older cattle which have not been re-exposed and have subsequently lost their immunity. Susceptible cattle introduced into the area from non-endemic areas would be subject to exposure and clinical disease.

The role of anaplasmosis and babesiosis in the "Secadera" complex needs to be clarified. Secadera was most frequently reported as the major single disease problem on the 37 ranches and is related to occur most frequently during the dry season in association with nutrition stress. The syndrome is characterized by a continual loss in body weight accompanied by anemia. Anaplasmosis and/or babesiosis are related to be present in nearly all cases and are considered to be important causal factors in association with nutritional stress.

B. microplus ticks were identified on each of the 37 ranches visited in the Llanos and were nearly equally distributed within the study area as indicated by the nonsignificant difference in tick counts between the 5 study zones. The infrequent occurrence of the other 3 species of ticks identified indicated that their importance as vectors or potential vectors of anaplasmosis and/or babesiosis is limited. The effect of annual climatic variation, such as occurs during the wet and dry seasons, on tick infestation of cattle in the Llanos needs to be determined to accurately assess the possibility of babesiosis due to instability in the vector population. Unstable epidemiological situations may be expected when tick populations are either naturally or artificially reduced to such low levels that the frequency of transmission is insufficient to maintain infection in young animals when passive immunity and natural resistance is highest (6).

North coast study

The 83 per cent prevalence of *Anaplasma* reactors in the pregnant cows and the 100 per cent infection of all 112 calves tested in the North coast study indicated that anaplasmosis is endemic on the 4 ranches included in the study. Kuttler, Adams and Zaraza tested 151 cattle for anaplasmosis at the ICA Turipaná Experimental Station, which is centrally located in relation to the 4 ranches included in the study, and reported 91 per cent reactors (8). The results reported by Kuttler, et al., and the results from the 4 ranches indicate the high prevalence of anaplasmosis in the area and further suggest that anaplasmosis is highly endemic in the North coast region.

The mean age of infection of the calves with anaplasmosis and babesiosis (11 weeks), indicated the calves were infected at an early age when maternal antibodies and natural resistance provided maximum protection against the development of severe clinical disease. The occurrence of only 2 cases of "apparent" clinical disease in the calves; the low *Anaplasma* and *Babesia* parasitemias; and the minimal decreases observed in the mean PCV's indicated the calves were not severely affected by the infections and that recovery was rapid.

The 100 per cent infection of the 107 calves with *B. bigemina* suggested that babesiosis due to infection with *B. bigemina* was endemic on the 4 ranches studied and the 57 per cent reactors observed in the 120 pregnant cows tested was probably lower than the true prevalence of infection. The low number of reactors in the pregnant cows was attributed to the failure of the CF test to identify subclinical carriers as reactors. Positive transmission tests with CF negative cattle (5) and the relatively short period of time following single infection during which the CF test will detect *B. bigemina* infection (10) indicated CF negative cattle may actually be subclinically infected.

Cauca valley study

The 71 per cent anaplasmosis prevalence and the 75 per cent prevalence of *B. bigemina* observed on the 6 ranches included in the study indicate that both anaplasmosis and babesiosis may be endemic in the Cauca valley. The distance of the 6 ranches from each other and the location of the ranches at the north, south and middle portion of the Cauca valley suggest a probably equal distribution of the diseases throughout the area.

The high prevalence of anaplasmosis and babesiosis in cattle greater than 24 months of age, 83 and 94 per cent respectively, indicated the importance of early infection of calves when resistance is maximum, and the probability of clinical infections occurring in

calves which are confined until 6 to 10 months of age when maternal antibodies have waned and natural resistance has decreased.

The danger of severe clinical infection occurring when susceptible cattle from non-endemic areas are introduced into the valley was substantiated.

The necessity is indicated of providing protective measures to calves, which must be confined until 6 to 10 months of age, and to susceptible cattle introduced into the valley from non-endemic areas.

Summary

The prevalence of *Anaplasma* reactors in the 3 study areas located in the Eastern plains, the North Coast and the Cauca Valley was found to be 75, 91 and 71 per cent respectively (Table 19). The prevalence of *B. bigemina* reactors in the 3 study areas was 42, 77 and 75 per cent respectively. The prevalence and even distribution of *Anaplasma* reactors in the Eastern plains indicates anaplasmosis is endemic within the entire study area. The prevalence of infection with *B. bigemina* in the Eastern plains indicates the area is endemic, however, the wide range in prevalence among the 37 herds studied (5 to 98) suggest the disease is not evenly distributed throughout the area.

The prevalence of *Anaplasma* and *B. bigemina* reactors on the 4 ranches on the North coast and the 6 ranches in the Cauca valley, though based on inadequate sample sizes for the regions in general, suggests that anaplasmosis and babesiosis are probably endemic in both regions.

The importance of exposing calves to infection at an early age when passive immunity and natural resistance provide maximum protection against clinical disease, and the necessity of providing protection to susceptible cattle which may be brought into the 3 areas was indicated.

The 37 ranches in the Eastern plains are presently being revisited during the dry season and the herds retested to detect if changes in prevalence have occurred and to determine the incidence of anaplasmosis and babesiosis. Tick infestation is being reassessed to determine if the level of infestation is affected by annual climatic variation.

The serum samples collected in each of the 3 study areas are presently being tested for *B. bigemina* and *B. argentina* infection using the indirect fluorescent antibody test to determine if the prevalence of *B. bigemina* is higher than that indicated by the CF test and to determine the prevalence of *B. argentina* which is considered to be equally as important and widespread in Colombia as *B. bigemina*.

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THE DIAGNOSIS OF BABESIOSIS IN AUSTRALIA

D.F. Mahoney *

The Australian Environment

Three species of *Babesia* occur in domestic animals in Australia, two in cattle, *Babesia argentina* and *B. bigemina*, and one in dogs, *B. canis*. *B. canis* causes a very mild disease in dogs and is of minor importance. It has attracted little interest from researchers and I do not propose to discuss it. The cattle parasites are present in tropical and subtropical Australia in strict parallel with the distribution of their only vector on the continent, *Boophilus microplus*. *B. argentina* is responsible for over 90 per cent of the outbreaks of babesiosis and is economically more important than *B. bigemina*. Both parasites appear to be distributed evenly throughout the enzootic area.

Two factors control the distribution of *B. microplus*. They are (a) climate, and (b) legislation. In the North and East of the continent as far down as the southern border of the State of Queensland. The hatched area on Figure 1 defines the vector distribution. The chief factor that controls its movement towards the center of the continent is climate, assisted by disease-control laws which force stock-owners to cleanse their cattle of ticks to move them from tick-infested to tick-free zones. These laws prevent the spread of ticks deep into the free areas where they might survive long enough to cause outbreaks of tick fever. However, control by law has little effect on the to-and-fro movement of ticks which takes place in the vicinity of this line and which is the result of climatic variation. Within the enzootic zone there are about 9 million cattle, 30 per cent of the Australian herd. It is not uniformly tick infested, but is really a complex mosaic of zones of different infestation density resulting from factors as diverse as local climate and soil conditions, tick resistance of the cattle, stocking rate and methods of husbandry. Some localized areas such as the one shown in northern Queensland (Figure 1) are virtually tick-free.

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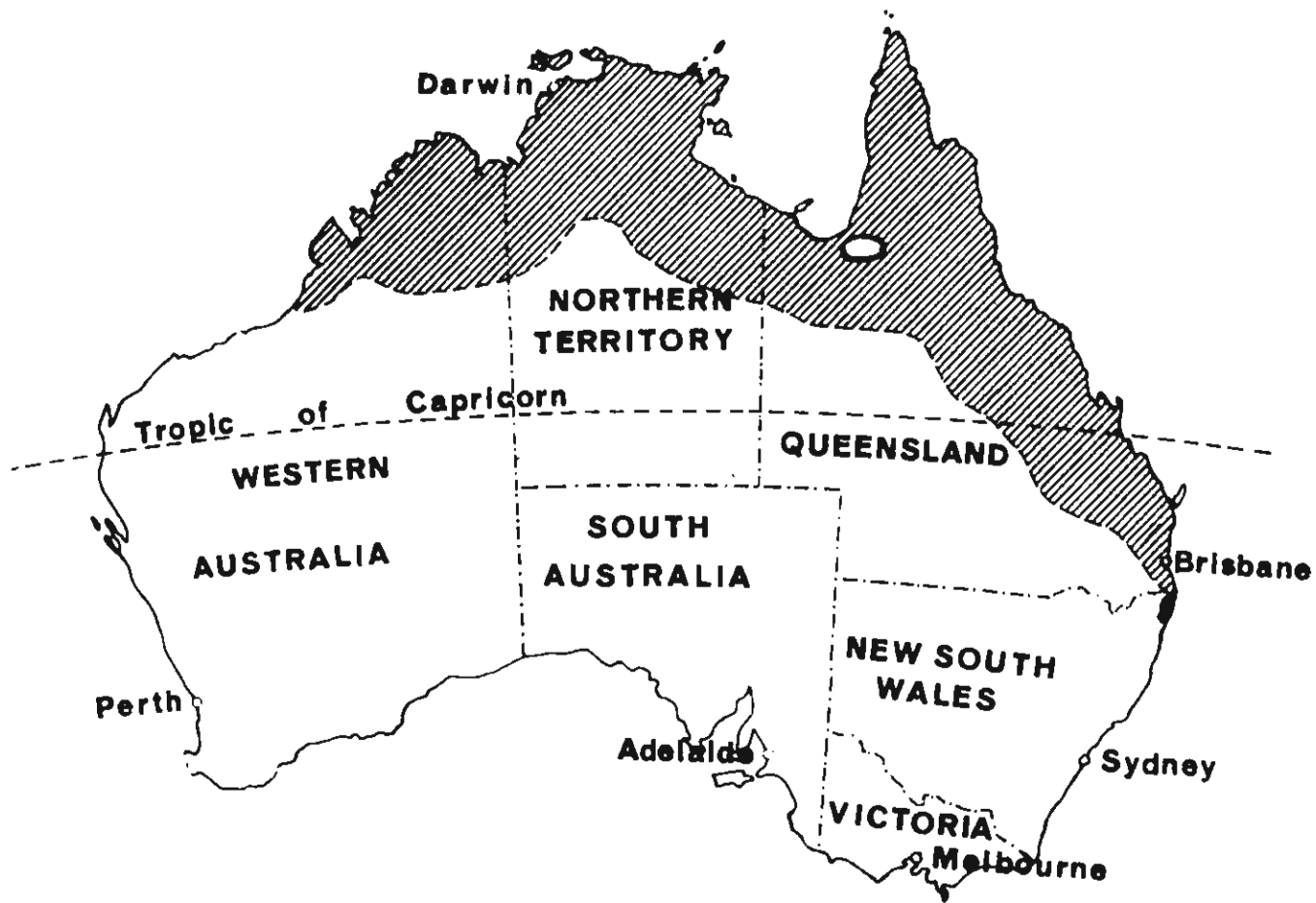


Fig. 1. Map of Australia showing the distribution of *Boophilus microplus*.

Husbandry methods vary in degree of sophistication. In the northern tropics properties are mostly large (size in square kilometers rather than hectares) and cattle are mustered about 4 times a year. Under these conditions, tick populations are influenced by climatic conditions rather than by the intensity of artificial control. Further South in subtropical and warm temperate areas the properties are smaller in size, the general standard of husbandry improves and vector control may be carried on at any desired level of intensity. In these areas, by applying well established control procedures, a stock owner may reduce tick burdens on his herd to a level such that the presence of the parasite is not obvious to the casual observer. Tick-control on properties, however, is undertaken on a voluntary basis.

The small area marked in black below the Queensland border is an area in which the vector has been almost eradicated. It marks the southern limit of tick infestation on the continent. It is a wet, warm temperate zone, highly favorable for tick reproduction. It contains 800,000 cattle which are maintained under strict quarantine precautions with respect to tick infestation and babesiosis. Tick populations have been very low in the area for many years and **Babesia** have almost disappeared from the indigenous cattle. The southward extension of the ticks is prevented by this quarantine area but the problem is that ticks and **Babesia** are being continually reintroduced from the enzootic areas to the North. Foci of infection containing either ticks and/or **Babesia** are immediately eradicated by acaricidal treatment and slaughter of infected animals.

The Diagnosis of Clinical Babesiosis

Recognition of the acute disease is of great importance in the situation I have just described. It occurs in a variety of circumstances:

- a) The introduction of animals from nonenzootic to enzootic areas.
- b) Emigration of animals from localized tick-free areas within the enzootic zone.
- c) Spread of ticks outside their normal geographical limits in abnormally favourable seasons.
- d) The accidental introduction of ticks to nonenzootic areas on travelling cattle.
- e) Local reduction of tick infestation in the enzootic area by artificial control followed by relaxation of control measures.
- f) Occasional occurrence in infected cattle due to breakdown of immunity on re-exposure.

Whenever a disease clinically resembling babesiosis occurs, the organism must be recognized as there are a number of other diseases with which babesiosis may be confused. The microscopic examination of smears of various types is therefore the cornerstone of diagnostic procedures for the acute disease. It is true that clinicians proceed with control measures on clinical evidence alone, but confirmation by smear examination is regarded as essential because it corrects mistakes in diagnosis that would otherwise discredit established control procedures. It also provides necessary data on prevalence for the benefit of disease control authorities. The examination of smears by individual veterinarians is not favored as their expertise in this area cannot hope to approach that of the specialist staff at diagnostic laboratories.

Smears are either of the thick or thin variety. Thin smears are taken from the peripheral blood and/or visceral organs. Thick smears are taken from peripheral blood. When we commenced to use thick films we had difficulty in observing *B. argentina*. It was found that Giemsa must contain a high proportion of Azure B in relation to the other Azure dyes in order to stain this parasite clearly. Satisfactory samples were characterized by a particular absorption spectrum (Mahoney & Saal, 1961) and it was then a matter of selecting suitable products from the range of Giemsa powders available commercially.

The following table (Anon, 1972) shows the smears recommended from the various types of field material.

Animals Available	Appropriate Smears
<p>Acutely Sick (i) animals</p> <p>(ii) 1 animal only</p>	<p>Thin smears of capillary blood from tail tip. From as many cattle as possible.</p> <p>Thin and thick blood smears.</p>
<p>Freshly Dead Animals No obvious decomposition</p>	<p>Thin blood smears from an extremity.</p> <p>Smears of the following organs in order of preference: Kidney Heart Muscle Spleen Liver Brain</p>

Animals in various stages
of decomposition

Thin blood smears from extremity.
Smears from following organs
in order of preference:
Spleen
Brain
Heart Muscle
Kidney
Liver

Subacutely sick animals
or recovering animals

Thin and thick blood smears
from as many cattle as
possible.

In the interpretation of smears the abundance of parasites is important. In smears from healthy tick-infested cattle, both **B. argentina** and **B. bigemina** may be seen. With **B. argentina** the numbers encountered vary between 1 and 100 per thick film and in thin films, one or two may be found after 15-20 minutes' search. In sick animals, about 1 to 10 parasites per oil immersion field are usually observed in thick films and about one every 5-20 oil immersion fields in a corresponding thin film. Similarly, parasite density in smears of organs should be considered. In the brain, for example, **B. argentina** may be found in small numbers in healthy carriers, but in sick animals the capillaries are either distended with infected red cells or at least contain numbers that are obviously significant. The finding of **B. bigemina** alone is often difficult to interpret as it rarely causes disease in Australia. It is implicated only if more than 1 per cent of the erythrocytes are infected and perhaps if smaller numbers are found accompanied by signs of anaemia. However, decisions on the significance of this parasite are always colored by the fact that it rarely causes disease.

Diagnosis in Relation to Epizootiology

The use of thick smears

The discovery that **Babesia** could be detected in thick films of the peripheral blood of healthy carrier animals (Mahoney, 1962) ushered in a systematic study of parasite prevalence in cattle. In the absence of reinfection, subclinical relapses of parasitemia were observed at intervals of several weeks in carrier animals over periods of 2-4 years. However, in enzootic herds exposed to continual reinfection, the prevalence of detectable parasitemia rose from zero at birth to 50 to 60 per cent between 1 and 2 years old and then declined as the animals aged. These curves of parasite prevalence by age-group always showed this general pattern even though different

levels of prevalence were observed. These results led to the conclusion that superinfection occurred in babesiosis and to the adaptation of an epidemiological model used in malaria for the analysis of the **Babesia** situation (Mahoney, 1969). The results suggested that the rate of **Babesia** transmission in permanently tick infested areas was of a low order, in the vicinity of once every 100-200 days for each individual animal, and foreshadowed later work on the dynamics of transmission which explained how herd susceptibility to babesiosis developed when tick burdens remained low (Mahoney & Ross, 1972).

Use of serological diagnosis

1) The Risk of Babesiosis

In common with other vector-borne diseases, babesiosis is a paradox for the stock-owner. Efficient control of the vector is economically desirable but in achieving this, the rate of transmission of **Babesia** is reduced below that required to maintain herd immunity. The development of a susceptible segment in a herd occurs unnoticed, because the stockowner has no way of knowing about it until the disease actually occurs too late for prevention. It is therefore a feared disease.

The dynamics of this situation depend on the following factors:

(i) Immunity after one infection lasts for years, and therefore susceptible animals are derived from one source only: calves growing up without exposure to infection (Mahoney, Wright & Mirre, 1973).

(ii) Calves are protected from clinical disease by several factors for variable periods after birth. The limit of the range is probably about 9 months. This is the maximum time for which calves should remain free of **B. argentina** infection in a tick infested environment.

(iii) For enzootic stability, infection has to occur at a rate sufficient to expose all calves at least once before 9 months of age. This rate can be expressed as an average daily probability. It is a simple mathematical exercise to show that the daily probability of infection for each animal in a herd has to be about 0.01 if all calves are to become infected by the time they are 9 months old (Mahoney & Ross, 1972). Actual probabilities of infection encountered in the field will be greater or less than this depending on the number of ticks in the environment.

Figure 2 shows how susceptible and immune segments develop in herds exposed to constant average daily probabilities of infection in the range of 0.0001 to 0.05. Each block diagram is produced by computer simulation of the occurrence of babesiosis in a herd of 100

calves from birth to 4 years of age. Each animal is exposed to the selected probability of infection each day, and depending on its age and the result of the exposure, it is placed in one of three categories infected before 9 months of age (light tone), infected after 9 months of age (dark tone), and not infected (white). The percentages show the proportion of the original group of 100 calves in each category after 4 years. The dark tone block represents the risk of occurrence of outbreaks of babesiosis and this reaches a maximum at probabilities in the vicinity of 0.001, and diminishes at lower probabilities.

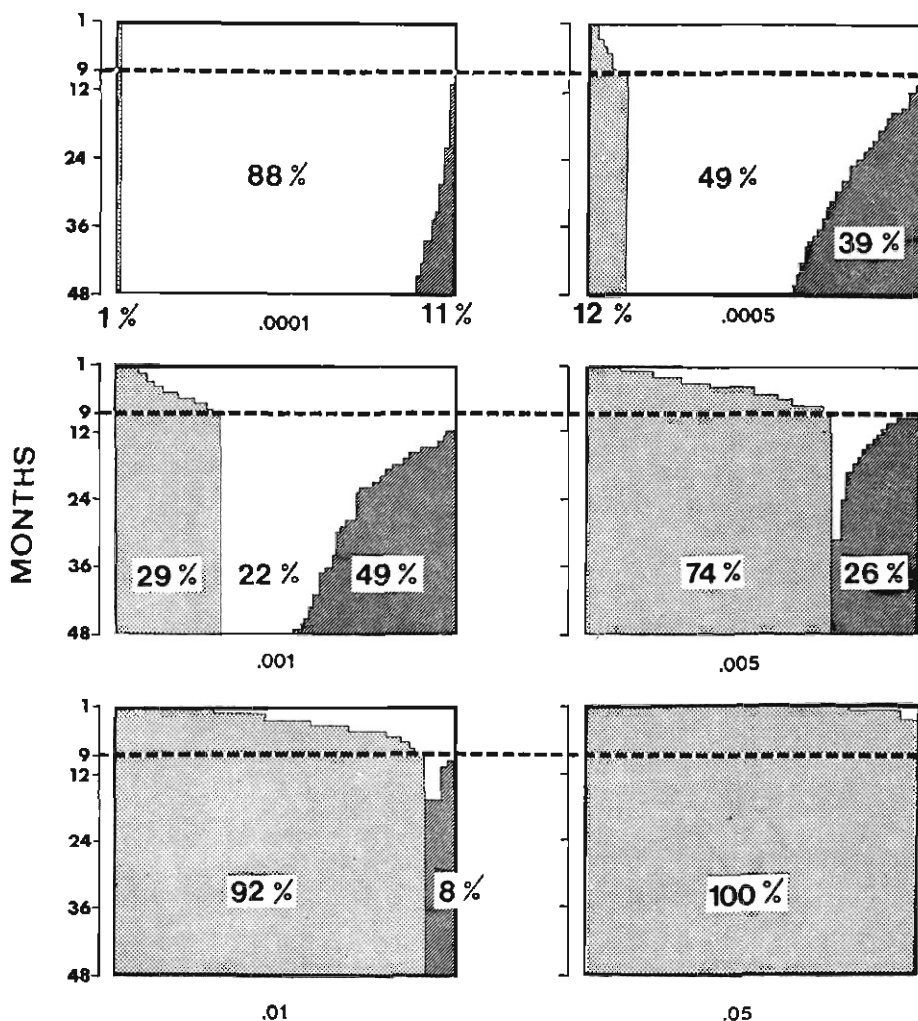


Fig. 2. Diagrammatic representation of how susceptible and immune segments develop in herds to constant average daily probabilities of infection with *Babesia argentina* in the range of 0.0001 to 0.05 (light tone = infected before 9 months of age; dark tone = infected after 9 months of age; white = not infected).

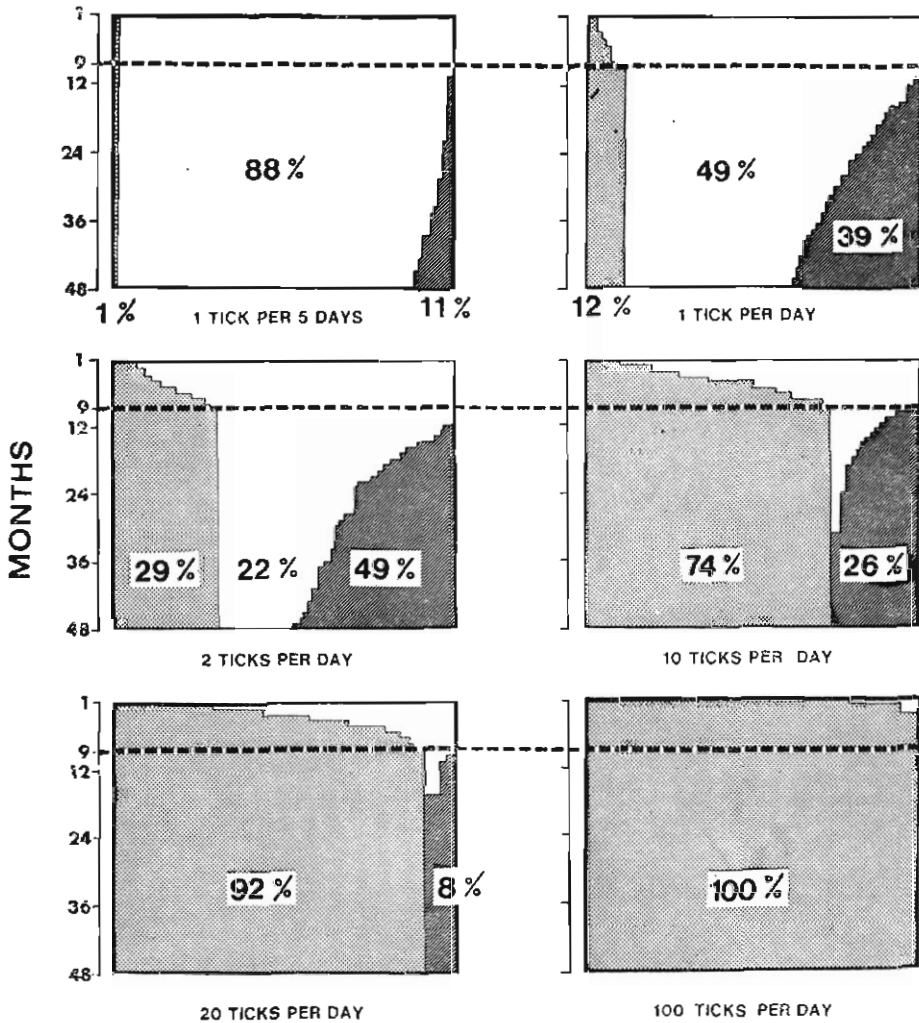


Fig. 3. The probability of infection with *Babesia argentina* converted to the equivalent number of daily tick bites for each animal (tone values the same as Fig. 2).

However, at these lower probabilities, the susceptible segment develops rapidly, but because the risk of clinical disease is low, a dangerous situation would develop without warning to an unsuspecting owner.

Figure 3 shows each probability converted to the equivalent number of daily tick-bites for each animal. The basis of this conversion was that the daily probability of infection may be represented by the number of tick bites/animal/ x proportion of ticks infected with *B. argentina*. Field observations in *B. taurus* herds have shown the latter to be of the order of 0.0005. These figures, linking level of tick infestation with the development of herd susceptibility, appeared to

hold in short term experiments with **B. taurus** cattle. **B. indicus**-cross cattle, however, appeared to require higher levels of tick infestation to produce similar rates of transmission to those observed in the **B. taurus** herds. For example, Figure 4 shows the tick infestation in three groups of experimental cattle maintained with different levels of tick infestation evaluated by weekly adult tick counts (Wharton & Utech, 1969). The percentage of the calves in each herd found to be infected with **B. argentina** at weaning (7-9 months of age) is also shown. The continuous-stocking and pasture-spelled groups were **B. taurus** breeds and their rates of babesial infection agreed well with the predictions in Figure 3. On the other hand, **B. indicus** cross cattle (i.e tick-resistant group) with 7 ticks/head/day showed no infection in calves at weaning although a similar level of tick infestation on **B. taurus** cattle would have infected between 30 and 70 per cent of the calves before 9 months of age. Obviously the babesial infection rate in the tick population propagating on the **B. indicus** group was lower than that on the **B. taurus** cattle.

These diagrams demonstrate that an important thing to know about enzootic babesiosis is the proportion of calves that become infected before they are 9 months of age. If this is 75 to 100 per cent, the epizootiological situation should remain stable. If less than 75 per cent, the disease is likely to become unstable, and vaccination is required to eliminate this risk. The only practical method for determination of infection rates in young cattle on a herd basis is a serological test. Our interest in serology of babesiosis in Australia arose from such considerations, first, as a method of research to investigate the dynamics of *Babesia* transmission and, secondly, as a practical means of helping stock-owners predict the risks from clinical babesiosis after tick-control programs have been applied. There has been a third more specialized use in the tick eradication area to eradicate subclinical infection from herds quarantined for tick infestation and/or babesiosis.

2) Evaluation of Serological Tests

(i) Complement fixation

The CF test employed a crude antigen. The only refinement was the concentration of parasitized erythrocytes from blood by the use of a hypotonic saline solution (Mahoney, 1967). **B. argentina** infected cells are more resistant to osmotic lysis than noninfected cells and 0.40-0.50 per cent salt solutions usually leave the infected cells intact. They may then be recovered by centrifugation. The CF antigen is prepared from the concentrated infected-cell suspension by lysis in distilled water and recovery of the parasite-stroma mixture by centrifugation.

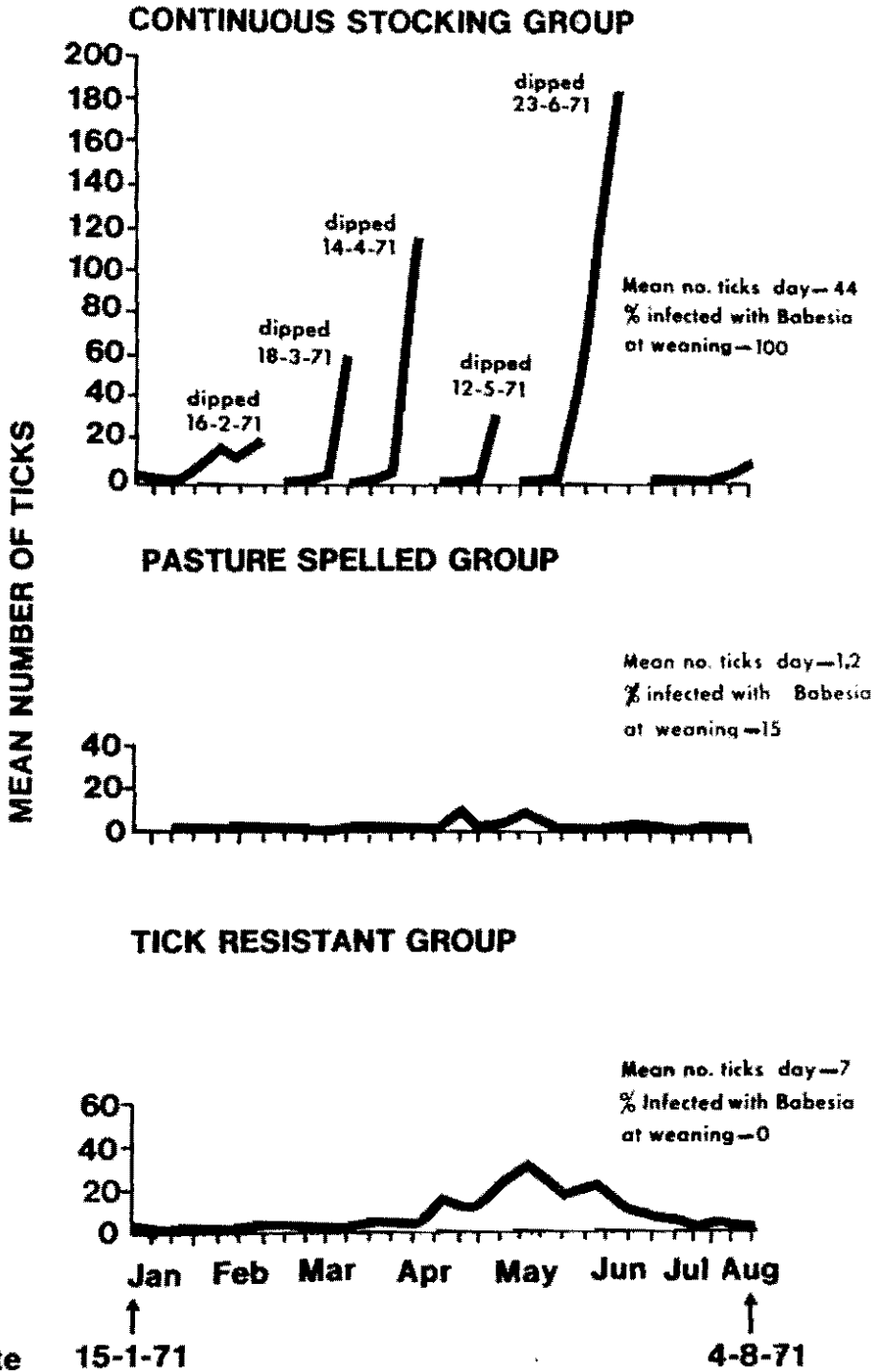


Fig. 4 The tick infestation in three groups of experimental cattle evaluated by weekly adult tick counts.

The characteristics of the CF test are: (a) about 2 percent false positive reactions occur in noninfected cattle (However, there is little cross reaction between species of *Babesia*); (b) fairly low sensitivity. The test is a reliable indication of subclinical infection for only the first 4-8 months of the carrier period. Antibodies fall below detectable levels before infection is lost (Mahoney, 1964).

The sensitivity of the CF test shows further decline in the enzootic situation because young animals, protected by colostral antibody, develop mild infection. Antibody formation is depressed first by the presence of passively transferred antibody and secondly by poor antigenic stimulation from mild infection. We have found the CF test of very limited value in our epidemiological investigations of enzootic babesiosis. Nevertheless in the eradication area of N.S.W. it was used extensively and effectively in surveys and also in the eradication of new foci of infection (Watts, 1969; Curnow, 1973a). Presumably this was because the situation was not enzootic and all infected cattle had experienced heavy antigenic stimulation and developed high levels of detectable antibody.

(ii) The indirect hemagglutination test

This test was tried first in the early sixties using crude soluble antigens, but in this form, was considered to have little advantage over the CF test (Curnow & Curnow, 1970). However, the purification of antigenic material from parasite extracts resulted in a marked improvement in its sensitivity and the test was developed for the routine diagnosis of *B. argentina* infection in our laboratory by 1970.

A choice of two serologically distinct antigens is available. One is obtained from the soluble material inside the infected erythrocyte which is released simply by lysis in distilled water. The other is obtained from soluble material released by sonic disintegration of a parasite-stroma suspension (Goodger, 1971). In both cases, the final purification step involves gel filtration on Sephadex G200 and recovery of antigen in the large molecular weight fraction present in the void-volume of the column. The percentage of false positive reactions in serum from noninfected cattle was originally in the high range of 2-8 percent. However, several routine procedures have reduced these reactions to about 0.5 percent. They are:

1. Serum is not allowed to stand overnight in the clot but removed after several hours. This is a common difficulty in testing field serum.

2. Prior to the test, serum is absorbed with the tanned and aldehyde treated sheep erythrocytes and also with a fraction prepared

from normal bovine erythrocytes by the same method as that used to prepare the babesial antigen.

In experimental infections with *B. argentina* in cattle maintained tick free under laboratory conditions, titers to the H.A. test persisted for years. Under these conditions, its sensitivity in detecting subclinical infection was 100 per cent and cross-reactions with *B. bigemina* antisera were confined to a short period after infection of the donor. In enzootic herds subject to continual tick infestation, however, we have occasionally obtained a negative result in a young animal that was shown to be infected by subinoculation of blood into a splenectomized calf. The incidence of such false negative reactions is 2.5 percent. Another source of error in field tests is the presence of colostral antibody which persists in calves from immune mothers for about 2 months after birth (Goodger & Mahoney, 1974a). With *B. bigemina*, evaluation of the test in experimental cattle has also demonstrated 100 percent efficiency in the detection of subclinical infection, but in herds in the enzootic area the incidence of false negative reactions in in cattle up to 12 months old has been high.

In our hands therefore the HA test is a highly efficient diagnostic method for *B. argentina* and is considered to be ideal for surveys of the incidence of this parasite, not only because of the high diagnostic efficiency but because of the large through-put of samples that can be achieved with the technique.

Unfortunately, similar efficiency in the diagnosis of *B. bigemina* in enzootic areas in Australia has not been obtained. This might be caused by the extremely mild nature of infection with this parasite which causes a weak antigenic stimulus in the host. It might also be true that the most efficient antigen has yet to be isolated.

(iii) Indirect fluorescent antibody test

The indirect fluorescent antibody test is also routinely used in the diagnosis of *B. argentina* infection in Australia. Its popularity emanates partly from ease of antigen preparation and preservation and simplicity of the technique. In tests designed to evaluate its efficiency in diagnosis it detected a high proportion of subclinical infection (97.6 per cent). Unfortunately it gave the highest overall incidence of false positive reactions in noninfected cattle (3.8 per cent) of the three laboratory tests (Johnston, Pearson & Leatch, 1973a). For this reason, combined with the comparative difficulty of performing large numbers of IFA tests at one time, the haemagglutination test is preferred to IFA for the diagnosis of *B. argentina* in our laboratory. A labeled anticomplement fluorescent antibody test has also been investigated and found to be as efficient

as the IFA test for the detection of *B. argentina* infections. It may be of use in testing feral animals for babesial infection because their specific antiglobulin reagents would not be available for IFA tests (Johnston, Pearson & Leatch, 1973b). The use of IFA technique for the diagnosis of *B. bigemina* has not been thoroughly investigated by any of the Australian groups presumably because of the lesser importance of this parasite.

(iv) Agglutination tests

Two simple agglutination tests have been developed in Australia. The first was for *B. bigemina* using a parasite-stroma suspension stained with Giemsa and preserved with formalin. It was inferior to the CF test as a general diagnostic method but showed evidence of strain specificity (Curnow, 1973b). Its potential as a method of analysis of antigenic variation of *B. bigemina* has never been fully explored.

A field test for the diagnosis of *B. bigemina* was developed as a direct follow-up to our epizootiological work described earlier which showed that the epizootiological status of a herd might be evaluated from a determination of the infection rate in calves at about 9 months of age. The laboratory tests were too cumbersome and expensive for this kind of disease control work. The test utilizes latex particles as the antigen carrier. It is performed on plasma obtained by using a portable centrifuge. Evaluated on laboratory infections its performance was superior to complement fixation in that subclinical infection was detected for up to 19 months after commencement and false positive reactions in noninfected animals were 2.8 per cent. However, in herds in the enzootic area that carried low tick burdens and contained a high proportion of young animals in the noninfected state the incidence of false positive reaction was high -12.5 per cent (Goodger & Mahoney, 1974b). Notwithstanding this difficulty, it is regarded as adequate as a herd test for the guidance of babesiosis control programs on properties suspected of being at risk but not yet experiencing a disease problem.

The infected cell agglutination test is a specialized method developed for the analysis of antigenic variation of *B. argentina*. It is of little interest as a diagnostic test because of marked strain specificity (Curnow, 1968). It relies on the fact that the parasites of each relapse of parasitemia carry a highly specific antigen which coats the surface of the infected cell. Each relapse population in the individual animal is different with respect to this antigen. However, we believe that this antigen/antibody system has something to do with protection and interest in this reaction is high at the present time.

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VACCINATION AGAINST BABESIOSIS IN AUSTRALIA

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In this paper I will give a very brief account of the history of babesiosis in Australia. This will be followed by more detailed information on the development of vaccination procedures. It will be convenient to present information on vaccination in two parts. One will deal with the approach used prior to 1964. The other will deal with the "modernization" of the methods which began in 1964 and has continued up to the present time. The role of vaccination and its effectiveness in Australia will be discussed finally.

History of Babesiosis in Australia

Babesiosis came to Australia from Indonesia during the last century. The first recorded outbreaks of babesiosis were in 1880-81 near Darwin. During the next 10 years, the disease moved across Australia toward the areas of greater development in the East and by 1890 was causing losses in North-West Queensland. During the next 10 years babesiosis spread a further 1,000 miles South and East until by about 1900 it was established at Brisbane near the southern border of Queensland. The infection appeared to become very virulent during this time, and it has been estimated that 3,000,000 cattle died in Queensland alone. However, it was also a period of considerable advancement of knowledge of the condition. The Australian disease was identified with "Texas Fever" of North America. Australian investigators were quick to seize on the results of blood inoculation experiments and by 1897 protective vaccination was being practiced.

The disease also spread in a westerly direction from the original focus near Darwin, but did so more slowly, probably because northern areas of West Australia remained relatively undeveloped. Shortly after 1900 the great epizootic was over. Except in West Australia babesiosis had ceased to spread, probably because the vector *B. microplus* had by then occupied virtually all areas ecologically suitable for its survival. It did not become permanently established in regions

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either drier or cooler that lay to the south and west of what had now become an enzootic area for babesiosis.

The final piece of relevant history occurred in the 1930's when it was demonstrated that not one but two species of *Babesia* occurred in Australia. Until then losses were always attributed to *B. bigemina*, but after a small species called *B. argentina* by Legg was shown to be present, it was realised that this was, in fact, the major cause of clinical babesiosis in Australia.

Immunization Prior to 1964

Immunization enjoyed great popularity and success between 1897-1900 when it was used against the rapidly spreading disease. When the epizootic stopped, the need for vaccination diminished sharply because all cattle in the infested areas had either died or were recovered. Several years later, however, a need for vaccination arose again. Some of the cattle born after the epizootic were found to be susceptible due to enzootic instability. Other cattle that were being imported from tick-free areas of Australia to those northern and eastern regions where *B. microplus* was now firmly established also required protection. Immunization was performed either by lay persons on their own properties or, when especially valuable cattle required treatment, by trained staff at government laboratories.

In general, the carrier-donor system was used. Cattle were either artificially infected by inoculation or selected from a naturally infected herd. When the donor was no longer experiencing acute babesiosis, blood for vaccine was taken into citrate solution or was defibrinated. This "recovered" blood produced less severe reactions in recipients than "acute" blood. Doses of up to 5 ml were used. Prior to the realization that *B. argentina* was present in Australia one cannot be sure which parasites were provided by vaccine donors. Those held at laboratories probably carried *B. bigemina* alone for much of the time. It is certain, however, that donors held on properties and exposed to ticks would have transmitted *B. argentina* and possibly *A. marginale* as well. Whatever the status of the donors, the method was generally accepted over a very long period up to the 1950's. By about 1957 a troublesome incidence of vaccine failure had become the stimulus for laboratory investigations of the effectiveness of vaccination. It was quickly established that the 5 ml dose of blood did not always contain sufficient parasites to infect recipients. Variability occurred between donors, and it was also found that the infectivity of the blood of individual donors tended to fluctuate.

One can only speculate on the reasons that vaccine failure had not been considered a problem earlier. It is likely, however, that enzootic

instability increased appreciably during the 1950's due to reduction in the tick population. This was caused by a change-over from arsenical acaricides to the more efficient chlorinated hydrocarbons. The consequent, increased incidence of susceptible cattle would have increased the pressure on vaccine to protect, thus revealing its inherent deficiency.

Development of Methods Used After 1964

The most basic initial investigations in the development of the new vaccine were those of infectivity. After it had been shown that blood from carrier-donors was about 70 per cent effective when used under optimal conditions at the laboratory, dilution experiments using highly infective blood were performed to determine at what level infectivity was lost. Using ten-fold dilutions there was no sharp cut-off point. However, not all animals receiving 10^5 *B. argentina* subcutaneously became infected, and this information was used in the final determination of the dose.

The other useful initial observation was on the dose response. This was found to be linear, and allowed the onset of the reaction to be predicted, providing the number of organisms injected was known. For example, for current vaccine strains injected subcutaneously the relationship is approximately:

Parasites Inoculated	Time to reaction (Days)
10^8	6
10^7	8 - 9
10^6	11 - 13

As a result of these early observations, a vaccine containing 10^7 *B. argentina* was made, and users of vaccine advised that they should take precautions against severe reactions during the second week after inoculation, and particularly at about days 8 - 10. The dose was fixed at 10^7 parasites mainly because it was 100 times the dose at which infectivity started to be lost, and allowed considerable margin for error.

Preparation of Parasites

To obtain sufficient parasites to prepare highly infective vaccine it was obvious that primary parasitemias would have to be utilized. Prior to 1964, vaccine had always been prepared in cattle 1-2 years old with their spleens intact. The first, highly infective vaccine was produced in a splenectomized calf, a few weeks old. Blood containing

about 7×10^7 *B. argentina*/ml was collected from the jugular vein and diluted to 10^7 per dose. Serial passage of the strain through splenectomized calves was continued, each calf in the series being used as a donor of vaccine.

At various times between 1964 and now, certain modifications of the method were made as a result of continuing observations of the production and use of vaccine. Thus,

1. *B. argentina* became adapted during passaging in splenectomized calves with two observable effects:

a) Parasitemias became higher as the predilection of *B. argentina* for the visceral circulation appeared to decrease;

b) The virulence of the parasite for normal, that is, non-splenectomized cattle decreased.

2. When calves were infected in a standard way by injecting 10^9 parasites intravenously, these multiplied so that blood contained over 10^8 parasites/ml 4 days later, and was ready for collection.

3. As the demand for vaccine grew, many more parasites were required. These were obtained by canulating the carotid artery and performing an exchange transfusion. Calves survived this procedure, and it was found that further similar collections could be performed on subsequent days. At times more parasites were produced at second and third collections than at the first.

4. In an attempt to produce still more parasites from a single calf, corticosteroids were used as immunosuppressants. Higher parasitemias were produced, but the practice was eventually abandoned because its use appeared to be associated with two deleterious effects. These were:

a) A tendency for calves to die of an acute condition by intravascular clotting before vaccine could be collected;

b) Vaccine failure following the use of parasites exposed to cortisone, suggesting some modification of antigenicity.

5. After several years of passaging it was found that three strains of *B. argentina* were no longer transmissible by the tick vector. There has been no change in the immunogenicity of these strains which are now being used in vaccine in preference to transmissible ones.

Preparation of Diluents

Dilution is required to reduce the number of parasites to 10^7 /dose. A number of synthetic diluents, such as PBS, buffered citrate solution,

Alsever's solution and Tyrode's solution were tested; all were much inferior to citrated bovine blood in maintaining the infectivity of parasites. Plasma was comparable with whole blood and serum slightly less effective. As a result of this first series of tests, normal bovine blood was used as the vaccine diluent. About 5 years after the introduction of the new vaccine, hemolytic anemia was observed in the new-born calves of a small percentage of vaccinated cows. This resulted from the sensitization of the cows by injections of blood vaccine and the transfer of hemolytic antibodies in colostrum to their calves. One of the steps taken to deal with the problem was to reduce the quantity of erythrocytes in each dose of vaccine. A new cell-free diluent has now been used for several years. This contains 50 per cent bovine plasma mixed with a balanced salt solution. The work with diluents is continuing, the most recent modification being the addition of glucose which may become depleted in certain batches of plasma. Current testing is aimed at reducing the proportion of plasma in the diluent.

Maintenance of Infectivity

As stated above, the effectiveness of vaccine depends on its infectivity. Infectivity is retained for much longer periods when parasites are chilled than if they are held at room temperature. As a result of studies of parasite survival, the following methods of preparing and using vaccine were adopted.

1. Vaccine was chilled to about 4° C as soon as it was collected from the calf.

2. It was stored and used for up to 1 week and then discarded.

3. To counteract the death of parasites during storage, the proportion of infective calf blood used in the vaccine mixture was increased each day by a factor of 1.5. Thus, on day 7, ten times as much calf blood was used to provide 10^7 viable parasites as on day 0.

4. Vaccine was sent chilled to the field in a special ice pack. The dose volume was reduced from 5 ml to 2 ml to facilitate transport.

5. Farmers were advised to use the vaccine as soon as possible, to store it in the refrigerator whenever possible and not to keep it more than 7 days even when it was being stored under optimal conditions.

Control of Contamination

The most likely type of contamination of a fairly serious nature during vaccine production is with blood-borne agents including "wild" strains of *Babesia*, *Anaplasma marginale*, *Eperythrozoon*, *Theileria mutans*, *Trypanosoma theileri* and *Borrelia theileri*.

The most alarming prospect of contamination is with other specific viral and bacterial infections of cattle, such as, ephemeral fever in Australia, blue tongue in areas enzootic for that disease, salmonellosis and leptospirosis. The agent of bovine leucosis is a particularly dangerous, potential contaminant.

The most common but least serious form of contamination is with skin and air-borne organisms that may enter the vaccine during collection from the calf and during handling in the laboratory.

1. The measures taken to prevent contamination with other blood parasites are:

a) Purchase of vaccine cattle from districts not enzootically affected by tick-borne diseases;

b) Serological testing of vaccine animals for **Babesia** and **Anaplasma**;

c) Monitoring of blood films and rectal temperatures at the laboratory to detect infection developing in vaccine animals, prior to their use;

d) Selection of young calves that are unlikely to have been exposed to infections with **Eperythrozoon**, **T. mutans**, trypanosomes and spirochaetes;

e) Strict quarantine measures to prevent **B. microplus** becoming established in areas where vaccine animals are kept;

f) Isolation of vaccine animals away from animals infected with **A. marginale**.

2. Measures taken to prevent vaccine becoming infected with pathogenic bacteria and viruses are:

a) Purchase of healthy cattle and regular health checks including serological testing;

b) Hygienic precautions to reduce the spread of salmonellosis of calves; bacteriological testing of calves and vaccine when **Salmonella** is suspected of being present; use of strong healthy calves for vaccine production;

c) Continuous monitoring of white cell hematology to detect leukotic changes in animals used to provide blood for transfusion and plasma for diluent;

d) Suspension of vaccine production if infection of animals has been observed or is suspected.

3. Measures taken to prevent contamination during collection and handling are:

a) Use of sterile technique where possible and hygienic precautions at all stages of production;

b) Addition of antibiotics to the vaccine.

Over the last 10 years no major instance of contamination has been observed. In this time 11-1/2 million doses of vaccine have been distributed. On two occasions small batches of vaccine were discovered to have been contaminated with *A. marginale*. The owners of cattle inoculated with this vaccine were advised, the animals treated, and no losses occurred. Other undetected breakdowns in the system may have occurred, but there has not been a single report from the field suggesting any untoward effect due to contaminated vaccine. The greatest difficulty at the laboratory has been with salmonellosis. Bacteraemias have been detected at the time vaccine was being collected from calves. Production stopped for several days during an ephemeral fever epizootic, because adult animals used for diluent preparation were affected. All these animals are now serologically positive to this disease and are considered to be immune. In the more recent ephemeral fever outbreaks, diluent is stored for at least a week before use, during which time donors are watched closely for signs of sickness.

The Use of *Babesia bigemina* in Vaccine

It has been obvious for many years that *B. bigemina* causes relatively little economic loss in Australia. This organism was, however, included in vaccine prior to 1964. It was eliminated from the standard vaccine for the following reasons:

1. An experiment was performed in which three groups of cattle were immunized against *B. argentina* alone, and then exposed to natural babesiosis in three different regions of Australia. All 32 animals in the experiment became infected with *B. bigemina* almost immediately, indicating a high infection rate with this parasite in the ticks infesting them. However, not one of the animals showed any clinical signs of infection indicating the low pathogenicity of the parasite.

2. When *B. bigemina* is taken into the laboratory and maintained by blood passage, virulence increases. It was observed that cattle were suffering more acutely from vaccine reactions than from natural infections.

At the present time, *B. bigemina* rarely causes significant loss. If a serious outbreak occurs vaccine containing the parasite is specially prepared by infecting a splenectomized calf with frozen parasites of a

strain known to be in an avirulent phase. This vaccine is also used sometimes to vaccinate cattle being exported from Australia.

Field Use of Vaccine

Owners and agents place orders for quantities of vaccine ranging from 1 dose to several thousand. These are prepared individually from stored components and dispatched directly to the user. As more information on the epizootiology of babesiosis and on the immunity produced by vaccine has become available, recommendations for its use have been modified. For example, during the first years after 1964 it was considered necessary to revaccinate cattle at regular intervals. High levels of immunity were generally observed, but sometimes cases of natural babesiosis were seen in repeatedly vaccinated cattle. A more serious consequence was the hemolytic anemia of new-born calves, referred to earlier, which followed the hyperimmunization of cows with bovine blood. It was found that two vaccinations, the second with a strain of *B. argentina* different from that used at the first, produced a more substantial immunity than repeated vaccination with a single strain. It is now recommended that cattle at risk receive two vaccinations with different strains as early as possible in life, and preferably before they reach breeding age. This approach and the use of cell-free vaccine diluents appear to have reduced the incidence of hemolytic anemia to a low level.

Vaccination against anaplasmosis is also performed in Australia with a living vaccine based on a strain of *A. centrale*. When this is to be used, we recommend that it be included with the babesial vaccine for the first vaccination.

Present Status of Vaccination

The major role of the vaccine is to reduce losses in cattle born and bred in the enzootic area, but which fail to become naturally immune because of enzootic instability. It is also used to protect cattle being imported into the enzootic zone from tick-free areas and certain susceptible Australian cattle being exported to tropical countries.

How useful is vaccination? We now have evidence that vaccination reduces the incidence of babesiosis by well over 90 per cent. Firstly, in field trials of the efficacy of vaccination there are 16 times as many clinical cases of *B. argentina* in unvaccinated as in vaccinated cattle. Secondly, from diagnostic records it appears that clinical attacks occur 13 times more frequently in unvaccinated than in vaccinated animals.

The new vaccine was accepted to an unusual degree by the farming community in Australia. Before the change was made in 1964 about 100,000 doses were supplied annually. Four years later, the demand

had risen to 1,200,000 doses and has been maintained at slightly above that level up to the present time. Approximately 70 per cent is monovalent *B. argentina* vaccine, most of the balance being *B. argentina* mixed with *A. centrale*.

Finally, effective vaccines against tick-borne diseases provide a weapon against the vectors which may constitute a greater threat than the diseases they transmit. This is the case with *B. microplus* which is a problem in many tropical and sub-tropical countries. When owners are persuaded that their cattle can be protected by a vaccine, and not be dependent for their immunity on tick infestations they are more likely to cooperate in any plan to reduce tick numbers or even eradicate the pest.

ACARICIDE RESISTANCE IN *BOOPHILUS MICROPLUS* IN AUSTRALIA

*R.H. Wharton * and W.J. Roulston **

The development of acaricide resistant strains of the cattle tick has been a recurring phenomenon over the past 30-40 years, in Australia and South America where *Boophilus microplus* occurs and in southern Africa where the so-called blue-tick *Boophilus decoloratus* is the usual one-host cattle tick (Wharton & Roulston 1970). Arsenic was used successfully to control, and in some areas, to eradicate cattle ticks in Australia for nearly 50 years after its acaricidal properties were discovered about the turn of the century. It is still used in quarantine dips at the U.S.A. - México border and in some dipping vats in Queensland. Unfortunately the resistance that eventually developed in *Boophilus* to arsenic, developed much more rapidly to DDT, to BHC, toxaphene and dieldrin and to organophosphorus (OP) and carbamate chemicals. In order to appreciate why acaricide resistance is so serious a problem in Australia it is necessary to understand the condition under which *B. microplus* exists and the approaches that are adopted to alleviate its effects on cattle.

Boophilus microplus in Australia

The principles on which control are based are (a) prevention of live body-weight losses and/or death from the direct effects of engorging ticks and (b) prevention of clinical disease from infection of cattle with the tick fever blood parasites *Babesia argentina*, *B. bigemina* and *Anaplasma marginale*. A recent inquiry estimated that the annual cost of the cattle tick in Australia was \$42 million**; **direct costs** of \$5 million to Governments, \$9 million to cattleowners (including \$3.5 million for acaricides), **indirect costs** of \$27 million, (including \$18 million production loss due to the effects of the cattle tick, \$2 million deaths from ticks and tick fever, \$7 million reduced value of hides and \$1 million research costs) (Cattle Tick Control Commission Inquiry, 1973). Eradication would provide the only permanent solution but has never been a practical proposition since the cattle tick was introduced into northern Australia from southeast Asia over a century ago.

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** Figures in U.S. dollars.

In Asia *B. microplus*, *Babesia* and Zebu (*Bos indicus*) cattle have been associated for many thousands of years and a state of equilibrium between the host cattle and its parasites has evolved. Also, under most circumstances, the cattle live close to man in densely populated areas and are collected every night and housed either within or adjacent to human dwellings. They rarely carry large numbers of *B. microplus* and infestations of more than ten engorged females per animal appear to be uncommon (R.H. Wharton unpublished) and clinical disease due to *Babesia* and *Anaplasma* is not a problem, although it is in imported, non-immune, dairy cattle (Legg, 1959). This fact has been forgotten or overlooked in many Asian countries in recent years when cattle have been imported from tick-free areas.

In Australia, the association was, and still is, entirely different. The first contacts were in the Northern Territory, a sparsely inhabited area, where the cattle industry was essentially an annual harvesting of wild cattle, all or nearly all of which were of British (Shorthorn) *Bos taurus* origin. Stocking rates were of the order of 1 beast to 10 ha (25-30 acres) and the country was lightly timbered with a sclerophyll eucalypt-dominated woodland. Cattle raising was conducted under these conditions on vast areas of the North of Western Australia, Northern Territory and Queensland. They are now occupied by the tick and remain for the most part unchanged; cattle owners make only a crude estimate of their cattle numbers, based on an annual mustering for branding, castration and sale. Obviously these are not the only conditions under which cattle and *B. microplus* co-exist in Australia today. Table 1 shows the areas occupied by the tick and the numbers of cattle directly affected by its presence in the various States in 1972 (Cattle Tick Control Commission Inquiry, 1973). These areas range from the relatively unfavorable drier tropics discussed above, to very favorable wet tropics where cattle on improved pastures are maintained at a beast to 0.5 ha (1 acre) and cooler sub-tropics where winter temperatures limit tick survival and cattle may be on excellent

Table 1. Numbers of cattle present in areas infested by the cattle tick in Australia (1972).

Región	Cattle (Million)	Km ²	Area (sq. ml)
Queensland	6.2	920,000	(353,000)
Northern Territory	0.75	360,000*	(140,000)
Western Australia	0.67	400,000*	(150,000)
New South Wales	0.88	20,000	(7,000)
Australia	8.5	1,700,000	(650,000)

* Crude estimates.

pastures at a beast to 0.5 ha (1 acre) or on less productive mixed pastures and woodland at a beast to 2-4 ha (5-10 acre). In the context of tick control in Australia it is also important to recognize that labor is expensive and it is not unusual for an owner assisted by one, or perhaps two, stockmen to manage 2000 cattle on 6000 ha (15000 acres).

The first contacts between *B. microplus* and cattle in northern Australia were disastrous, mainly because of *Babesia*, but also because of tick control in Australia it is also important to recognize that labor breed cattle. Losses of 50-80 percent were not uncommon (Seddon, 1952) and, during the period from 1896 to 1903 when the tick was spreading and becoming established along the Queensland coast, cattle numbers fell from 6.8 to 2.5 million (Anon 1968-9). It is impossible to determine what proportion of this decline was due to ticks since this period coincided with one of the longest and most severe droughts in Queensland's history (Foley 1957). Comparable losses occurred among sheep which were unaffected by ticks but cattle owners attributed a great part of their losses to ticks and this has left a legacy of fear of ticks and tick fever that remains today.

Cattle that survive tick fever develop an immunity to reinfection and calves born by immune mothers do not show clinical disease provided they are infected by about 9 months (Mahoney & Ross 1972). Thus, tick fever is not a serious problem in enzootic areas, provided transmission is maintained at a satisfactory level and this may require a population of about 5-10 engorged female ticks per day on cattle (Mahoney & Ross 1972). These facts were unknown to cattlemen and scientists at the time the cattle tick was spreading. Their efforts were directed toward (a) developing a crude vaccine to protect uninfected cattle, (b) devising methods for killing parasitic ticks on cattle, and (c) limiting the southward movement of the tick to the more densely populated southern areas of Australia. They were moderately successful in two of their objectives, infected "bleeder" cattle served as a valuable source of crude vaccine for many years before the more reliable standardized vaccines were developed (Callow & Mellors 1966) and arsenic in dipping vats protected cattle from tick infestation for many years in Australia, Africa and the Americas. They probably also succeeded in preventing the cattle tick from reaching its southern limit on the New South Wales coast where, unhindered, the tick would probably survive at least 300 km (200 miles) further south than the existing quarantine line (McCulloch and Lewis 1968). This would place the southern limit at between 31 and 32° S which is similar to the southern limit in Brazil (Figure 1).

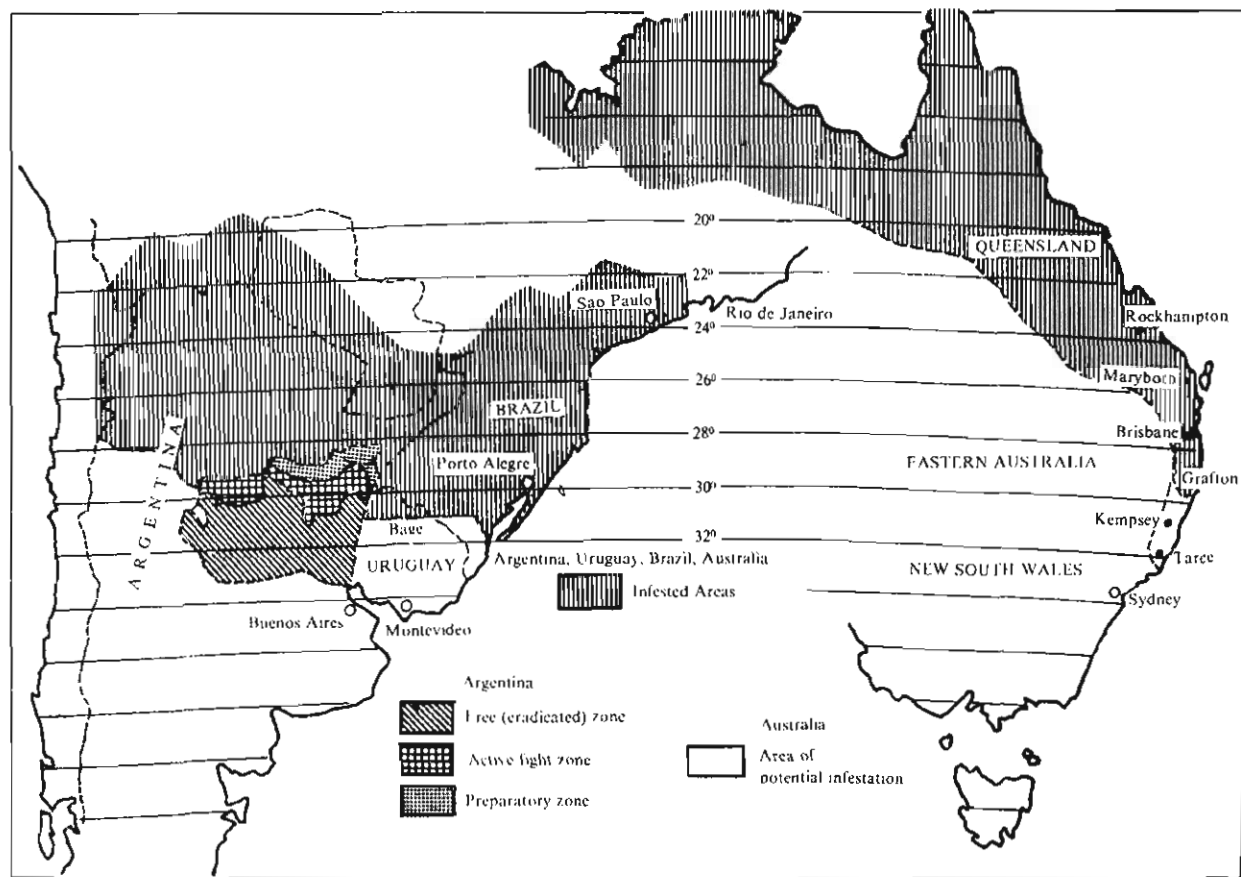


Fig. 1. Comparison of distribution of *Boophilus microplus* in Australia and South America.

Approaches to Cattle Tick Control

The attitudes and control policies adopted during the early years of the cattle tick in Australia determined to a very large extent the subsequent approaches to the problem by cattleowners and by government authorities.

In Queensland, and North Australia generally, there was no practical alternative to a policy of living with ticks and tick fever. Government participation has been concerned mainly with control over stock movements, particularly the movement of cattle from tick-infested to tick-free country; the development, production and distribution of tick fever vaccines; the registration of acaricides; monitoring for acaricide resistance; and with advice for cattleowners. The policy of living with ticks and tick fever coupled with (a) the belief stated by Riek (1965) that cattle "are immune to tick fever for varying periods of time and under field conditions immunity is maintained by constant reinfection with infected ticks" and (b) a cattle industry based until recent years almost exclusively on British breed cattle, led cattleowners to rely on acaricides to "manage" their tick populations at a satisfactory level i.e. too low to cause obvious harmful effects but high enough to ensure maintenance of tick fever immunity. The usual management practice has been for the cattleowner to learn by experience how frequently cattle require acaricidal treatment and this may vary from 4-5 times per year in southern Queensland to 6-8 times in central and 10-12 in northern Queensland. This approach is the most practical for the cattleowner but is expensive in terms of labor and acaricide and has failed because the cattle industry has been based on European (*Bos taurus*) cattle. When acaricide-resistance occurs the cattleowners may have to treat his cattle at weekly or fortnightly intervals and the frequent mustering becomes an intolerable burden. With the development of a highly efficient vaccine, tick fever due to *Babesia* can be removed as a threat and some cattle-owners on well-developed properties are now willing to reduce and keep tick numbers at very low levels. However tradition dies hard and many still like to see a few ticks on their cattle because this indicates that the cattle are retaining their immunity.

Management strategies that take advantage of weak links in the life system e.g. strategic (planned) dipping or pasture spelling have special applications but have failed as general control measures in Queensland because of the varied conditions of management—pasture, cattle and human—that prevail (Wharton 1973). Cattle that are resistant to ticks, expressed by their ability to prevent large numbers of ticks from maturing, are the most attractive and logical approach and have reduced the tick to an insignificant problem (as in Brazil)

on many cattle properties in northern Australia. The fact that resistance is associated primarily with Zebu cattle was the main reason why this approach to tick control was not adopted earlier. A major change in cattlemen's attitudes occurred in the 1960's and Zebu x British cattle now dominate in tropical areas. The change in attitude has yet to occur in the majority of cattlemen in the subtropical areas where the most severe problems of acaricide resistance exist.

In New South Wales, eradication has always been the objective. Relatively small campaigns have succeeded in areas marginal for tick survival but the largest and most ambitious campaign in 1956-7 failed (Mackerras et al. 1961). Tick and tick fever control have been maintained for many years at a very high level. A government authority directs and finances control; it provides dipping facilities, instructs cattlemen when and where to dip their cattle, and rigidly controls stock movements related to gazetted Tick Quarantine Areas and from Queensland to New South Wales. This approach arose from fears that the tick would occupy all the cattle areas of eastern Australia and the consequent efforts taken to prevent its southward spread. A double fence was constructed at the N.S.W. Queensland border and remains guarded to prevent the introduction of ticks from Queensland because of the different policies north and south of the border. To the north, tick fever is enzootic and cattle may be expected to be infected at some time with *Babesia*. To the south, tick control is maintained at such a high level that tick fever is not normally transmitted (Curnow 1973). Some 880,000 non-immune cattle within the quarantine area are protected from the threat of ticks and tick fever by a "strategic dipping" program at 3-week intervals in the spring and summer. This has resulted in only 40-50 tick infestations and 3.4 tick fever outbreaks being found per year in the 8000 cattle holdings in the quarantine area over the past 7-8 years (Cattle Tick Control Commission Inquiry, 1973). It is believed that the majority of these tick infestations and tick fever outbreaks originated in Queensland.

Development, Recognition and Significance of Resistance

Resistance has developed to all acaricides that have been used extensively against *B. microplus* in Australia. However, the rate of development and its significance in relation to control have varied with the type of acaricide and the way it has been used. Table 2 shows that resistance has developed more rapidly in Queensland than in New South Wales indicating that a policy of "living with ticks and tick fever" based on the uncontrolled and often haphazard application of acaricides produces resistance more rapidly than the "maximal

Table 2. Records of acaricide resistance to *B. microplus* in Australia relative to the type of acaricide, period of use and region where resistance developed.

Acaricide	Year	Year resistance recognized	
	Introduced	Queensland	N.S.W.
Arsenic	1895	1937	1952
DDT*	1946	1954	Nil
BHC**	1950	1952	*
Diazinon***	1956	1963	1969
Dioxathion***	1958	1963	1969
Coumaphos***	1959	1966	1970
Chlorpyrifos	1966	1970	1974

* Resistance to DDT is linked with resistance to pyrethrins (Shaw, Cook and Carson 1968).

** Resistance to BHC is linked with resistance to toxaphene and dieldrin; they were introduced independently for tick control in Queensland and resistance developed independently (Wharton and Rouston 1970). They were not used in N.S.W.

*** A number of OP-chemicals were introduced at about the same time in Queensland and resistance to some but not all of the early introductions were linked. Dioxathion was the only OP-acaricide used initially in N.S.W. and was not used widely until 1962.

control" practiced in New South Wales (there is also a strong probability that the majority of the resistant ticks that have been found in N.S.W. were migrants from Queensland). It shows also that whereas arsenic was used successfully for about 40 years before resistance developed, resistance to DDT was recognized about 8 years after it was first used, to BHC-toxaphene-dieldrin after less than 2 years and to the OP-acaricides after about 7 years.

Organophosphorus (OP) resistance has created a fascinating and frustrating challenge to all associated with the cattle industry. OP-acaricides were marketed for the first time in 1956 but were not used widely until about 1960, yet by 1974 eight resistant strains had been recognized. Each is distinct toxicologically and biochemically and at least two resistance mechanisms are involved, cholinesterases that are relatively insensitive to inhibition by OP-acaricides and detoxification systems that metabolize the chemicals to non-toxic metabolites. Some strains combine both mechanisms.

The History

1962-3: Control failed in central Queensland and a commercial company reported resistance in laboratory tests in England (Shaw & Malcolm 1964). Similar failures became apparent in the same and other areas and Australian authorities confirmed the resistance in laboratory tests and undertook spraying trials to determine the

significance of resistance in relation to control (Roulston et al. 1968a). Biochemical studies in England and Australia showed that although the resistant ticks exhibited reduced cholinesterase (AChE) activity, resistance was due to decreased sensitivity of the tick AChE (Schuntner et al. 1968). Because strains from several areas demonstrated similar properties it was decided to name the resistance after the locality where it was first recognized. Thus the name Ridglands came into scientific literature. Resistance extended to a wide range of OP and carbamate chemicals and four out of the six commercial acaricides available at that time were rendered ineffective. These were dioxathion (delnav), diazinon, carbophenothion (trithion) and carbaryl (Sevin). Resistance to the remaining two coumaphos (co-ral) and ethion was low.

1966: A new type of resistance was recognized at Biarra in the Brisbane Valley and the ticks exhibited increased resistance to all OP-chemicals including ethion and coumaphos. A number of candidate acaricides that had been shown to be effective against Ridglands ticks were also eliminated. Resistance to chlorpyrifos (Dursban), bromophos ethyl and phosmet (Imidan) was low and these acaricides were introduced to control Biarra ticks (Roulston & Wharton 1967). Resistance was again found to be due to decreased sensitivity of AChE to inhibition, but more of a less sensitive enzyme was present in Biarra ticks and was the reason for the increased resistance (Roulston et al. 1968b). Spraying trials at about this time demonstrated the potential of chlordimeform (Chlorphenamidine, C8514) as an acaricide (Roulston & Wharton 1967).

1967: Resistance was recognized at Mackay in north Queensland. The ticks at first appeared to be the same as Biarra because of the wide spectrum of resistance including resistance to ethion and coumaphos. Subsequent toxicological and biochemical studies showed that the ticks differed from Biarra but for most practical purposes they were the same. A major difference was the resistance mechanism which was shown to be detoxication (Roulston et al. 1969).

1970: New types of resistance were found that had developed in response to the introduction of chlorpyrifos and bromophos ethyl to control OP-resistant ticks (a) ticks exhibiting very high resistance to chlorpyrifos were recorded from three localities in south-eastern Queensland. At first it appeared that the increased resistance was associated principally with chlorpyrifos because laboratory tests did not demonstrate significant changes in resistance to bromophos ethyl; spraying trials subsequently demonstrated a marked deterioration in efficiency of both acaricides; ticks of this so called Mt. Alford strain of ticks have AChE with similar properties to Biarra AChE but have

added detoxication as a resistance mechanism (b) ticks exhibiting high resistance to chlorpyrifos were recorded also from Gracemere in central Queensland (O'Sullivan & Green 1971); this strain is an extension of Ridgeland, adding detoxication to decreased AChE sensitivity (Schnitzerling et al. 1974).

1971-5: Over the period from 1964-1970 an efficient monitoring service, supported by laboratory and field evaluation of existing and candidate acaricides provided bases for accurate diagnosis of the type of resistance and for advice on the most efficient acaricide to use. With extremely high levels of resistance and an even increasing complexity of resistant strains both services have become difficult to maintain.

The new types of resistance that have been recognized in central and northern Queensland include (a) Bajool, characterized by resistance to chlorpyrifos, (b) and (c) Ingham and Tully from the wet tropics, distinguished by a lack of resistance to diazinon, low levels of resistance in laboratory tests that are not correlated with control failures in the field and a greater AChE activity than in susceptible ticks. All depend on detoxication. A disconcerting change has occurred also in Mackay ticks which appear to have substituted decreased AChE sensitivity for part of the detoxication that was the resistance mechanism originally; ticks with similar characteristics have been found in the field and the strain has been renamed as the Mackay-Silkwood. Table 3 (after Roulston & Nolan 1975) summarizes the main features that distinguish the different strains and a summary of the resistance factors to a variety of acaricides is included as Appendix 1. It should be noted that the strains are characterized after culturing in the laboratory to eliminate the susceptible component that is present in almost all field samples; the resistance factors were determined by exposure of larvae to the acaricide in oil-impregnated filter paper packets (Stone & Haydock 1962, Anon. 1973).

Efficiency of Acaricides Against Normal and OP-resistant Ticks

Difficulties in control have increased with the emergence of highly resistant strains. Ridgeland ticks were controlled comparatively easily and Biarra ticks were controlled effectively, though expensively, by chlorpyrifos and bromophos ethyl. When enhanced resistance to these acaricides developed in Mt. Alford ticks, the only OP-acaricide left was the unstable phosmet (Imidan), used as a spray. Cattleowners were dipping their cattle 10-12 times a year instead 4-5 and still unable to achieve satisfactory control. The addition of chlordimeform to several OP-acaricides produced satisfactory mixtures, again with problems of stability, but provided a short breathing space for the

Table 3. Characteristics of OP-resistant strains of *B. microplus* in Australia relative to the Yeerongpilly susceptible reference strain and classified according to the level of AChE, the insensitivity of the AChE to oxons, the metabolism expressed as the percentage of oxon and total hydrolytic products present six hours after treatment with 0.003% coumaphos and 0.001% chlorpyrifos.

Strain	Mean resistance to five OP acaricides	Level of AChE activity Yeerongpilly = 100	AChE insensitivity Yeerongpilly = 1			Metabolites (%)		total hydrolytic products	total hydrolytic products
			coroxon	diazoxon	chlorpyrifosoxon	(0.003%) Coumaphos	(0.001%) Chlorpyrifos		
Yeerongpilly (Reference susceptible)	1	100	1	1	1	21	47	2.6	27
Ridgeland (1963)	4	21	10	30	10	24	41	—	—
Biarra (1966)	27	42	940	132	84	16	56	2.7	26
Mackay (1967)	6	58	1.3	1	—	1	95	—	—
Mackay-Silkwood (1970)	7	20	4	39	7	4	75	2.6	80
Gracemere (1970)	56	21	10	35	4	—	—	0.5	38
Mt Alford (1970)	92	47	1115	132	76	18	47	0.4	39
Bajool (1972)	10	14	1	18	4	—	—	0.6	26
Tully (1972)	3	155	10	4	4	2	86	10	54
Ingham (1973)	3	180	13	3	3	2	89	0.3	44

development of alternative acaricides. Several satisfactory acaricides have now been marketed and additional candidate acaricides are being evaluated. However, these are more expensive, or more difficult to manage or are less efficient than a range of OP-acaricides were before resistance developed.

The decline in efficiency of OP-acaricides in relation to the Ridgелands, Biarra and Mt. Alford ticks is illustrated in Figure 2 and a summary of these data together with additional data on the efficiency of past, present and candidate acaricides against susceptible and resistant strains is included as Appendix 2. The evaluation of acaricidal efficiency is based on spraying trials in which groups of 3 calves are artificially infested three times a week for 3.5 weeks, then sprayed, and the survival of ticks following treatment is estimated from the numbers and weight of eggs produced by engorged females

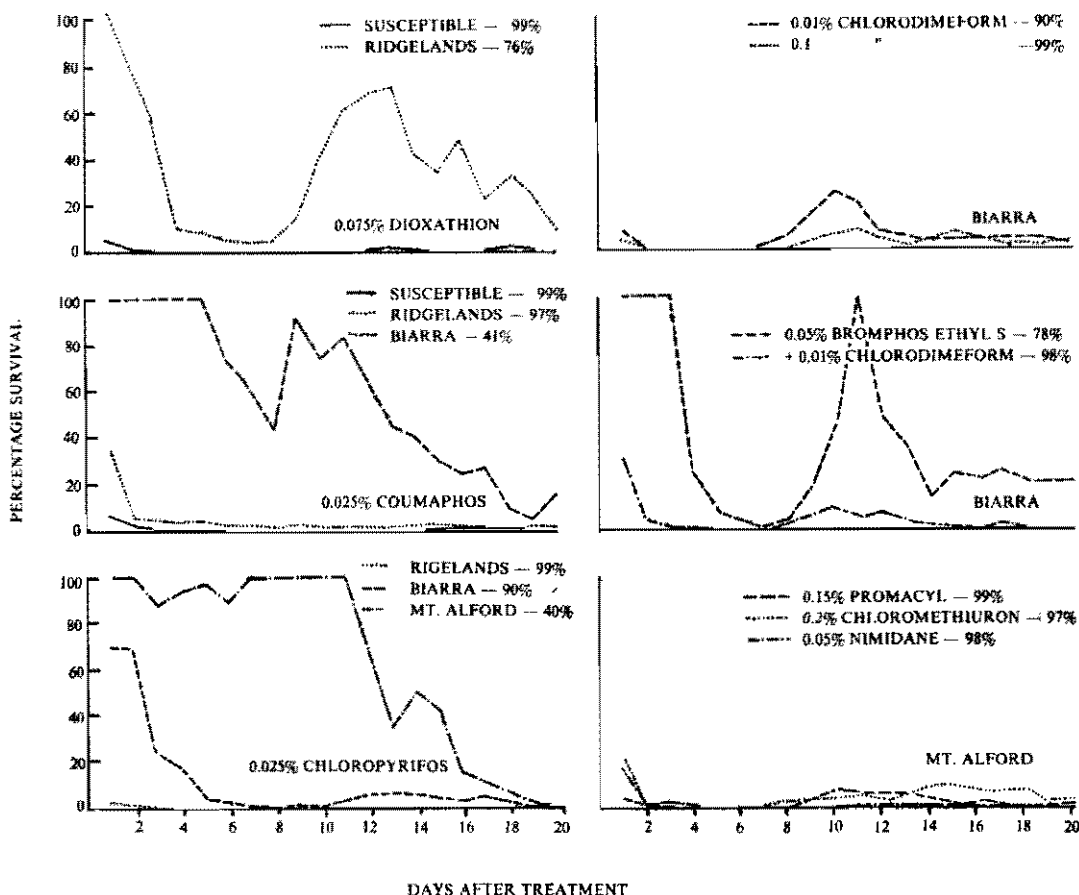


Fig. 2. The changes in efficiency of acaricides against *Boophilus microplus* in relation to the development of organophosphorus resistance.

that dropped from sprayed and unsprayed groups before and after treatment: the efficiency may then be expressed as the mortality over the parasitic life cycle of 22 days (Roulston et al., 1971).

The main features are as follows:

a) All OP-acaricides as single treatments produced more than 99 per cent mortality of normal ticks and were more efficient than arsenic (77%) or DDT (93%).

b) The effect of resistance may be very great or only minor e.g. chlorpyrifos produces 99% mortality of Ridglands, 90% of Biarra but only 40% mortality of Mt. Alford ticks.

c) The concentration of acaricide is very important particularly when dealing with resistant ticks e.g. bromophos ethyl S at 0.05% produces 78% control of Biarra compared with 97% if used at 0.1%.

d) No modern acaricide can consistently produce the level of control formerly achieved by several OP-acaricides*. Chlordimeform at 0.1% is the most consistent but produces only 97% mortality of Mackay and needs to be buffered in an acid dipping vat to maintain stability. Promacyl (carbamate) clenpyrin (iminopyrrolidine) and chloromethiuron (thiourea) will control Mt. Alford ticks but at increased cost.

e) Amitraz (triazapentadiene) and nimidane (dithietane) are potential commercial acaricides; amitraz is unstable unless buffered at $> \text{pH } 10$.

f) Biarra and Mackay ticks are highly resistant to arsenic: a proportion of Biarra and Mackay ticks are also resistant to toxaphene and a proportion of Gracemere and Bajool ticks are resistant to DDT.

In other trials it has been shown that the addition of piperonyl butoxide will restore the efficiency of carbaryl against OP-resistant ticks (Schuntner et al. 1974): pyrethrum and piperonyl butoxide is a highly effective but expensive and unstable mixture, and the synthetic pyrethroid NRDC143 is highly effective (Roulston & Wharton unpublished).

Distribution, Incidence and Development in Relation to Acaricidal Use

Resistance has never been universal and its development is clearly related to the degree of selection pressure. Arsenic was still being used successfully on some cattle in 1966 and DDT was apparently generally effective in 1962 when it was banned because of unacceptable residues despite the emergence of resistance in 1955 (Newton 1967). Similarly

* Amitraz was registered in 1975 and produces excellent control of all strains.

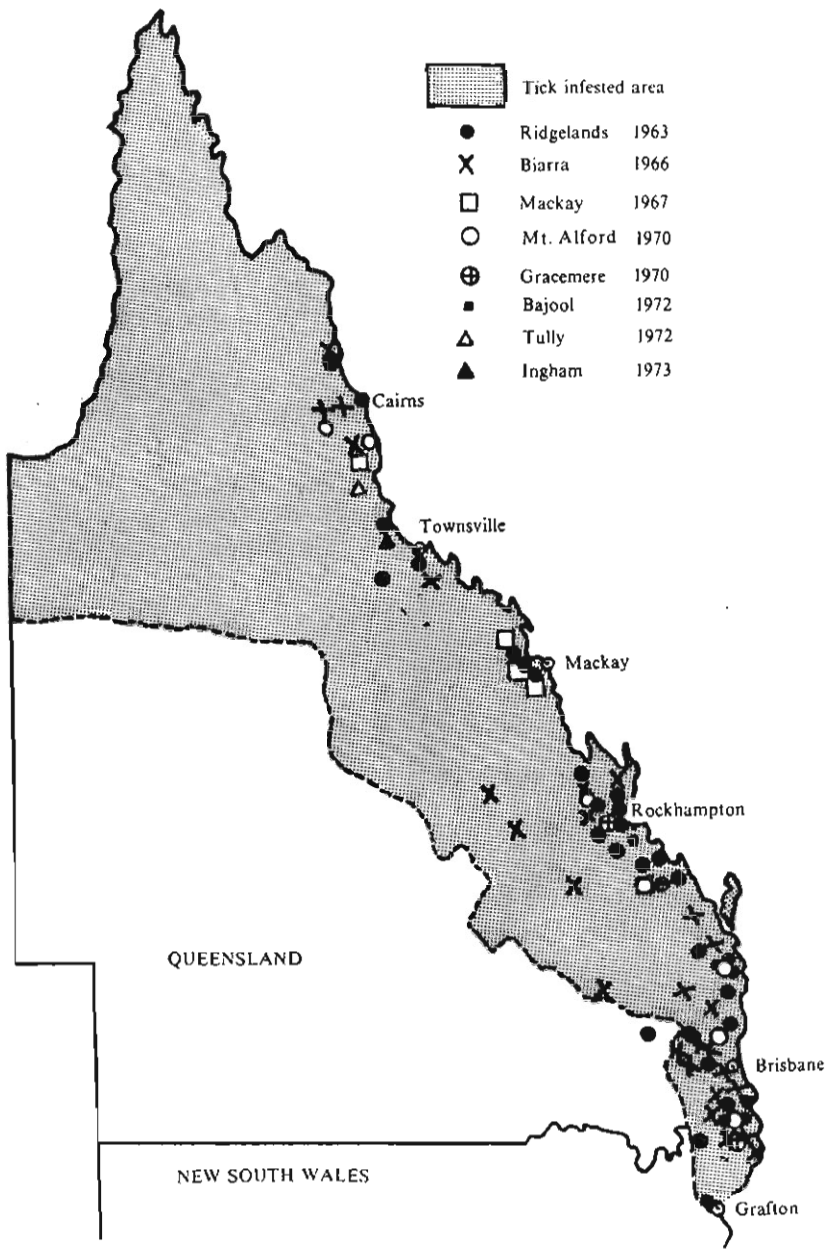


Fig. 3. Distribution of OP-resistance in *Boophilus microplus* in Australia.

dioxathion, coumaphos and ethion are used successfully in many areas in Australia despite evidence of resistance in some areas for a considerable period.

Figure 3 shows the areas where the various types of OP-resistance have been recorded in Australia. Resistance is more common in the more densely populated coastal areas where cattle populations are higher and acaricides are used more frequently; it is less common in the more sparsely populated areas but appears to be spreading and has been recorded on properties where acaricides are used only 6-8 times per year. For several years after resistant strains were discovered there was little evidence of their movement or development elsewhere. Thus Biarra was restricted to south-eastern Queensland and Mackay to northern Queensland. Now different types of resistance are being diagnosed in the same locality and this adds to the difficulties of accurate identification of field samples. The Queensland Department of Primary Industries established a diagnostic service in 1964 and Table 4 summarizes the diagnoses made over the 1964-1974 period. It is not feasible to determine the exact proportion of cattle holdings that are infested with resistant ticks but it is estimated that there are about 16000 cattle holdings in tick infested areas of Queensland and the total of over 3000 diagnosed cases of resistance indicates the widespread significance of the problem.

Ridglands OP-resistance was found in New South Wales for the first time in 1969, followed by Biarra resistance in 1970 and Mt Alford and Gracemere in 1974. Since 1972 almost all infestations found there have been of the Biarra-type (Cattle Tick Control Commission Inquiry, 1973).

Table 4. Diagnoses of OP-resistance in field samples of *B. microplus* recorded by the Queensland Department of Primary Industries 1964-1974 (P.J. Green unpublished 1975).

Year	No. Submitted	No. resistant (%)	Ridglands	Biarra	Mackay	Grace-mere	Mt Alford
1964-5	49	30 (61)	29	1	—	—	—
1966-7	463	220 (48)	74	145	1	—	—
1968-9	1860	884 (48)	432	400	52	—	—
1970-71	1694	991 (59)	410	500	35	7	39
1972-74	1605	1201 (75)	309	729	50	11	102
Total	5671	3326 (59)	1254	1775	138	18	141

It has been suggested that the intervals of dipping adopted in Australia are more conducive to the emergence of resistance than the very short 7 day intervals used in southern Africa (Shaw 1970). It is also a commonly accepted view in Australia that the inefficient use of acaricides at less than the recommended concentration is the main reason for our problem (Newton 1967). A recent meeting of an FAO panel discussed the "Relation between acaricidal dosage, frequency of application and biology of ticks and the development of resistance". It was noted that resistance to arsenic, DDT, and BHC-toxaphene acaricides developed at about the same rate in *B. decoloratus* in South Africa and in *B. microplus* in Australia and South American countries, but organophosphorus resistance has developed much more rapidly in *B. microplus*, particularly in Australia. Thus rate of development has differed with different acaricide groups under conditions of weekly (South Africa) and much greater intervals of 3-5 week or more (Australia, South America). The only general principles are (a) resistance develops more rapidly where the tick is under a constant acaricidal challenge aimed at maintaining sufficient tick numbers to maintain *Babesia* immunity in cattle, and (b) resistance develops more slowly in two and three-host ticks whose generations extend over a year or more compared with one-host *Boophilus* species in which four or more generations occur in one year" (Anon 1973).

There is no scientific evidence to show what conditions are most favourable for the emergence of resistance. However, circumstantial evidence supports the view that the very efficient application of acaricides, that results in very low populations, delays resistance. There are two examples, N.S.W. (above) and Rhodesia where much more stringent regulations apply and where the first report of resistance was to arsenic in 1963, 25 years after resistance had occurred in neighbouring South Africa (Jones-Davis 1972). It is also relevant that the major resistance problems in *B. microplus* appear to have arisen in Australia, Argentina and Brazil in areas where cattleowners have attempted to "manage" tick populations on *Bos taurus* cattle by the use of acaricides. They have not arisen in Asia where Zebu cattle, *Babesia* and *B. microplus* evolved or where, as in large areas of Brazil, a similar equilibrium has been restored. In 1971, the cattle population in Brazil, almost all in areas favorable to the cattle tick, was estimated to be 85 million and the acaricide market \$1.5 million; the Australian market for 7-8 million cattle in tick infested areas was about \$3 million i.e. twice the market for one-tenth the cattle. The difference in Brazil was the predominance of *Bos indicus* type cattle except for the southern areas, where their cattle industry was based on Hereford and Angus cattle and where they had similar acaricide-resistance problems to those that plague the Australian cattle industry.

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Appendix 1. Resistance factors of unfed larvae of *OP*-resistant strains of *Boophilus microplus* to organophosphorus carbamate and other acaricidal chemicals, determined by comparison with the LC50's for unselected larvae of the reference Yeerongpilly strain (Roulston & Wilson unpublished).

Chemical	Yeerongpilly Susceptible	Ridgeland 1963	Biarra 1966	Muckay 1967	Gracemere 1970	Mt Alford 1970	Bajool 1972	Tully 1972	Ingham 1973
Organophosphorus									
bromophos ethyl	0.21	1	4	1	1	5	1	2	2
coumaphos	0.039	1	48	9	4	>48	1	6	5
chlorfenvinphos	0.021	5	15	20	6	16	4	4	2
chlorpyrifos	0.023	2	6	2	44	110	7	1	4
cyanophos	0.011	7	141	2	8	160	8	1	2
diazinon	0.012	11	50	10	228	260	37	1	2
dimethoate	0.0011	1040	420	300	1600	460	1400	3	7
dioxathion	0.12	8	18	14	12	54	9	6	2
ethion	0.18	3	38	9	3	35	3	6	3
phosalone	0.095	5	7	21	6	6	7	6	6
phosmet	0.018	2	3	7	2	2	7	1	1
Carbamate									
carbaryl	0.0022	9	9	25	16	11	12	2	2
promacyl	0.069	3	2	4	4	2	4	1	1
Formamidine									
chlordimeform *	0.00074	1	1	2	1	1	1	1	—
amitraz *	0.0046	1	1	1	1	1	1	1	—
Thiourea									
chloromethiuron *	0.0015	1	1	2	1	1	1	1	—
Iminopyrrolidine									
clenpyrin	1.5	1	1	2	2	1	—	1	2
Dithietane									
nimidane	0.7	—	—	—	—	1	—	—	—

* Exposure period increased to 144 hours; recent tests indicate that it will be preferable to enclose the packets in plastic bags and expose larvae for 48 hours.

98 Appendix 2. Mortality of *B. microplus* estimated from spraying trials in which stalled calves infested with normal or OP-resistant ticks were sprayed with past, present or candidate acaricide. Evaluation by this method gives slightly better results than under normal dipping conditions.

Chemicals	Conc. %	Yeerongpilly Normal	Ridge-lands 1963	Biarra 1966	Mackay 1967	Gracemere 1970	Mt Alford 1970	Bajool 1972	Tully 1972	Ingham 1973 . .
Miscellaneous										
Arsenic	0.2	77	78	14	21	—	—	—	—	—
DDT	0.5	93	—	91	—	—	—	—	—	—
Pyrethrum 1	0.025	>99	—	—	—	—	—	—	99	—
Organophosphorus										
bromophos	0.05	>99	97	78	92	—	60	98	89	78
ethyl S ²	0.1	>99	99	97	—	99	67	—	97	—
chlorpyrifos	0.025	>99	99	90	99	82	46	92	99	83
coumaphos	0.025	>99	99	41	89	88	—	—	89	78
dioxathion	0.075	>99	76	41	68	—	—	—	92	—
ethion	0.075	>99	99	74	90	99	34	—	90	93
phosmet 3	0.075	>99	99	96	88	99	95	98	95	—
Carbamate										
carbaryl	0.2	>99	99	75	—	—	—	—	92	—
promacyl	0.15	>99	—	98	95	98	99	94	95	99
Formamidine										
chlordimeform 4	0.1	>99	99	99	97	99	99	99	>99	>99
amitraz	0.025	>99	—	>99	>99	—	>99	>99	—	—
Thiourea										
chloromethiuron	0.2	97	—	—	99	97	97	93	97	95
Iminopyrrolidine										
clenpyrin	0.3	97	—	99	85	99	98	97	91	99
Dithietane										
nimidane	0.05	—	—	—	99	—	98	96	99	99

1) Applied as 1:10 mixture with piperonyl butoxide. 2) Applied as a mixture of 0.05% bromophos ethyl and 0.006% chlorfenvinphos. 3) Unstable and used only as a spray. 4) Unstable unless buffered to acid salt; also effective in mixtures with OP-acaricides eg. chlorpyrifos + 0.01% chlordimeform = 94% mortality of Mt. Alford, or, bromophos ethyl S + 0.01% = 98% mortality of Biarra.

Addendum

ACARICIDE RESISTANCE IN LATIN AMERICA

Boophilus microplus is widely dispersed in Latin American countries and is the most important tick infesting cattle in the area extending from Mexico to Argentina. Resistance developed to arsenic and to the organochlorine chemicals, DDT, BHC and toxaphene in Argentina, Brazil, Venezuela and Colombia (Wharton & Roulston 1970) but there has been little published evidence of resistance to the organophosphorus (OP) acaricides.

1. Shaw, Cook and Carson (1968) reported the presence of Ridgeland type resistance from Maracaibo, Venezuela, and from Brazil.

2. Fluck and Rufenacht (1969) reported the collection of the "Las Guerisas" strain from the province of Entre Ríos, Argentina in 1964; the ticks were resistant to diazinon and dioxathion and the resistance appeared to be greater than Ridgeland but less than Biarra.

3. Torrado and Gutiérrez (1970) described the "G" (Goya, Corrientes) strain from Argentina with low resistance to a number of OP-acaricides but high resistance to coumaphos (Asuntol).

4. Amaral et al. (1974) described the D (Rio Pardo, Rio Grande do Sul) and M (Leopoldina, Minas Gerais) strains from Brazil with increased resistance to coumaphos, dioxathion and ethion.

5. Additional information was provided by R.D. Shaw and P.L. Crampton of the Cooper Technical Bureau for the FAO/OIE Ad Hoc Consultation on Control of Protozoal Tick-borne Diseases of Cattle at Nairobi in 1972. OP-resistance was reported from Argentina, Brazil, Colombia and from Venezuela. Most diagnoses indicated Ridgeland-type resistance (found for the first time in South America in 1963 in **B. microplus** collected from Santa Ambrosina, Rio Grande do Sul, Brazil). A different type of resistance was found at Guaimarito in Perijá, Zulia, Venezuela in 1970 and subsequently at two other cattle properties in Zulia and on two properties in Valle, Colombia. Ticks of the Guaimarito strain exhibit high resistance to dioxathion, ethion and coumaphos but not to chlorpyrifos or bromophos ethyl. Shaw & Crampton (loc. cit.) described these ticks as having a "super-Ridgeland" resistance i.e. higher than Ridgeland but less than Biarra.

Comments

It is probable that OP-resistance is present in all Central and South American countries where acaricides have been used frequently to

control **B. microplus**. México may be an exception since there have been no reports of resistance from that country. It is clear that Ridgeland-type resistance has developed in a number of countries but it is not possible to relate the other types of resistance to those that have been identified in Australia. Methods of testing have differed but Shaw & Crampton (loc. cit.) suggest that resistance of the Biarra-type had not been identified in Brazil, Venezuela and Argentina. Resistance to chlorpyrifos which is a characteristic of the Gracemere and Mt Alford strains in Australia had developed in Rio Grande do Sul, Brazil in 1971 (Wharton, 1974) but again the relationship with Australian types of resistance is not known.

It would be most useful to characterize the OP-resistant strains of Latin America. A tentative method for the detection of resistance in **B. microplus** has been recommended by FAO, based on methods used by Australia workers (FAO, 1969). The documentation of resistance would allow government authorities and the cattleowners to apply the knowledge developed in Australia so that a rational choice of alternative acaricides can be made, when and where OP-resistance develops.

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DIAGNOSIS OF ANAPLASMOSIS: A REVIEW

*K.L. Kuttler **

In their early publication (1893) on *Babesia bigemina*, Smith and Kilborne (39, 40) described a small deep staining coccus-like, marginal body in erythrocytes. They considered these bodies as a phase in the life cycle of *B. bigemina*, but suggested the possibility that a second blood parasite might be responsible. Theiler, (41, 42) in 1910, recognized these marginal points as the causative agent of an entirely separate disease entity, which he entitled anaplasmosis or gall sickness.

These bodies stain a deep purple with the Romanowsky stains, when observed with a light microscope. Observations of the causative organism by this and other methods is still basic for positive diagnosis. In addition to these methods, *Anaplasma marginale* has been observed by a variety of methods including: fluorescent antibody (35, 36), phase microscopy (6, 27) and acridine orange (11); electron microscopy including ultra-thin sections (5, 7, 8, 14, 32, 34), indirect fluorescent antibody (21), and with new methylene blue (38).

Observations of *Anaplasma* by one or more of these techniques is useful and plays an important role in the diagnosis of anaplasmosis, but ordinarily the period of time in which *Anaplasma* bodies are sufficiently numerous to be diagnostic is short. The infection is known to persist for extended periods of time beyond the acute phase, during which observable parasitemias are absent. Parasitemias probably do exist, and with techniques such as acridine orange and fluorescent antibody the chances of finding the organism in chronic infections are improved, but even so are below an acceptable level of confidence for diagnosis in these instances.

Hence, a reliable serologic technique assumes importance in the diagnosis of anaplasmosis and becomes essential in control programs based on identifying and removing carrier infections.

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Complement Fixation

Rees and Mohler (31), in 1934, described the preparation of two specific complement-fixation (CF) antigens. One was prepared from laked blood, which was centrifuged and washed, while the other was prepared from extracts of ticks previously fed on *Anaplasma* infected animals. Consistency in the production of CF antigens was not achieved until 1949 when Mohler, et al., (23) and Mott and Gates (25) described the production of a CF antigen from the blood of animals acutely infected with *Anaplasma*. This technique involved the collection of blood with a high parasitemia, washing the erythrocytes in physiological saline (PSS), and lysing them in 30 volumes of cold distilled water saturated with CO₂. The resulting precipitate was collected and washed in PSS by centrifugation. In 1952, Price, et al., (29) prepared a CF antigen, which in some respects represented an improvement. Highly infected erythrocytes were washed in PSS, and then lysed in distilled water. Particulate matter, cell stroma and *Anaplasma* bodies were then recovered by passing the lysed cells through a Sharples centrifuge at 40,000 x G. The sediment was collected, washed, and re-suspended in saline. Separate evaluation of both the CO₂ and the water lysed antigens by Gates (12), has shown each to be effective. A standardized CF antigen (9, 10) was prepared in quantity using a combination of both techniques, and until recently was available from the USDA, Beltsville, Maryland.

A new, more purified CF antigen involving the use of the French pressure cell is now being produced and distributed by the USDA, primarily for use in a micro-plate system (2). At Texas A&M, we have been using a micro-plate system for the CF test since 1968, using the old standardized USDA antigen. The titer of this antigen can be increased about three times by sonication, and used at 1:30 to 1:38 dilutions as compared to 1:9 and 1:12 in unsonicated antigen. In addition to using greater antigen dilutions, sonication disrupts most of the particulate matter thus facilitating the use of the antigen in the microsystem dropper and dilution loops. The correlation of results between the micro-system and the standard tube test is good.

The CF test has been thoroughly evaluated under many conditions and by numerous investigations (13, 15, 16, 17, 23, 28, 30, 37). The general consensus is that this test has an accuracy of about 95.96%. This test has been used successfully for a number of years in field control programs, where it has been necessary to identify and remove carrier animals (26, 22). Positive CF tests can result in the absence of infection, but only under artificial conditions. It has been shown that calves nursing *Anaplasma* infected cattle will become positive

due to the ingestion of colostral antibodies (20). The injection of killed *Anaplasma* antigen plus adjuvants will also elicit a positive CF test (18).

The techniques involved in performing the CF test are described in a publication by the USDA (1). Basically, all reagents - complement, hemolytic amboceptor, and antigen- are standardized by titration so that two units of each are used in the test proper. A 2% sheep erythrocyte suspension is used in the hemolytic system. Veronal buffer (VB), pH 7.4, is used as diluent throughout the test. A 1:5 serum dilution is used for the screen, and complete fixation (4+) of complement at this level is considered positive. The following protocol is followed:

1. 0.1 ml serum + 0.4 ml VB to give a 1:5 dilution.
2. Diluted serum inactivated at 58° C for 35 minutes.
3. After serum has cooled thoroughly.
 - a) 0.5 ml antigen diluted in VB to contain exactly two units is added.
 - b) 0.5 ml complement diluted in VB to contain exactly two units is added.
4. Serum - complement - antigen - incubated 60 minutes at 37.5° C.
5. 1.0 ml of hemolytic system is added, tubes are mixed and incubated 45 minutes at 37.5° C. The hemolytic system is composed of equal parts 2% sheep erythrocytes, and VB containing hemolytic amboceptor calculated to give two units.
6. After the final incubation, the tubes (now containing 2.5 ml total volume) are centrifuged five minutes at 2000 RPM on a clinical centrifuge (International PR-2) and read immediately.
7. A reading of 4+ shows no hemolysis.

3+ -	25% hemolysis
2+ -	50% hemolysis
1+ -	75% hemolysis
Tr. -	>100% hemolysis
Negative -	100% hemolysis

Serum titrations are made in the same way except for the use of two-fold serum dilutions in VB, usually extending from 1:5 to 1:1280 or higher. Serum titers in excess of 1:1280 are rare.

The micro-test is conducted by our laboratories in essentially the same way except that the quantities are about 1/20 the amounts used in the tube test. Dilutions are made by the diluting loops, and the 0.025 ml amounts added by calibrated droppers. All titration of reagents are accomplished using a tube technique as described (1).

The protocol as published by the USDA (1) calls for the use of phenolized serum samples. Phenol is added in 0.5% concentrations as a serum preservative. The suggestion has been made that this step will enhance the antibody response. In an attempt to verify this and to quantitate any possible change, a series of tests were made on phenolized and non-phenolized sera and compared statistically.

Sera from 47 animals known to be infected with *A. marginale* were collected cell free and divided into two aliquots. One was left unphenolized; the other phenolized to a final concentration of 0.5%. All sera were then tested 18 hours later for evidence of CF antibodies. Sera from twelve cattle known to be *Anaplasma* negative were similarly treated and tested. All sera were tested at a 1:5 dilution and categorized as positive, suspicious, and negative as described previously.

The results are presented in Table 1. Phenolized and non-phenolized serum samples from the twelve non-infected calves were all negative. Of the phenolized samples taken from infected cattle, 83% were positive and 17% were suspicious; none were negative. In the non-phenolized samples only 62% were positive, 26% were suspicious, and 12% were negative.

A second trial involving serum titrations was conducted on 25 sera from cattle thought to be infected. Four of the 25 had received a sterilizing treatment of oxytetracycline, and were gradually losing

Table 1. Comparison of response of 1:5 dilutions using phenolized (0.5%) and non-phenolized serum.

	No. of Animals	Phenolized			Non-Phenolized		
		Pos.	Susp.	Neg.	Pos.	Sus.	Neg.
Known Anap. Pos.							
Serums	47	39	8	0	29	12	6
Specificity		83%	17%	0%	62%	26%	12%
Known Anap. Neg.							
Serums	12	0	0	12	0	0	12
Specificity		0%	0%	100%	0%	0%	100%
Totals	59	39	8	12	29	12	18

Table 2. The influence of added phenol on prozone activity in titrations of serum samples taken from 25 intact 18-24 Mo. Old heifers supposedly infected with anaplasmosis.

	Serum Dilutions				
	1:5	1:10	1:20	1:40	1:80
Avg. Reaction					
Phenolized	3.0	2.66	1.66	0.72	0.28
Avg. Reaction					
Non-Phenolized	1.85	2.12	1.68	0.78	0.32
% Agreement	62%	80%	99%	92%	88%
Significance	P<0.01	P<0.01	NS	NS	NS

titer. A wide range of serum titers were present. Serum dilutions ranging from 1:5 to 1:80 were tested on all animals. Reactions were averaged on the basis of 1+, 2+, 3+, and 4+ reactions. An analysis of paired difference was conducted to determine whether or not significant differences did occur.

The results are presented in Table 2. At the 1:5 and 1:10 dilutions agreement between phenolized and non-phenolized samples was 62% and 80% respectively. The reactions at these dilutions were significantly ($P<0.01$) more intense in the phenolized samples. There were no significant intensity differences in the 1:20, 1:40, and 1:80 dilutions, and the agreement was 99%, 92% and 88% respectively.

The pronounced tendency for prozone reactions in non-phenolized samples was greatly reduced by the addition of phenol, which appeared to intensify the reactions at the lower dilutions.

Capillary Tube Agglutination Test

In 1963, a capillary tube agglutination test (CA) was developed (33) and for a time was available commercially*. This antigen is more highly purified than the old USDA CF antigen. It is prepared from heavily parasitized erythrocytes, lysed by sonication and separated from erythrocytic stroma by differential centrifugation. The test is relatively easy to perform. A capillary tube is filled to about 1/3 capacity with antigen and then filled with serum which has been inactivated thirty minutes at 56° C. The tubes are placed upright in

* Diamond Laboratories - Des Moines, Iowa.

Table 3. Serologic examination of 501 cattle comparing the CF and capillary-tube agglutination test.

Complement-Fixation	Capillary-Tube Agglutination	% Agreement
Pos. 210 (41.9%)	218	89.71%
Susp. 33 (6.6%)	—	—
Neg. 258 (51.5%)	283	89.75%
Overall agreement		89.73%

molding wax, sealed at the exposed end to prevent drying and then allowed to stand at room temperature. Readings are made 24 hours later as negative, 1+, 2+, and 3+, depending on the degree of visible clumping. Welters and Zuschek (43) described this test as being equal to, or possibly more sensitive and specific than the CF test, with the advantage of being less costly and less complicated to conduct. In one trial of 501 comparisons between the CF and CA tests, there was an 88% agreement (20) (Table 3). This trial gave the impression that the CF test was slightly more sensitive in picking up early reactions than the CA test. As with the CF test, animals given killed *Anaplasma* antigens developed positive CA reactions (20). CA reactions were also seen in calves nursing *Anaplasma* infected cattle (20). Analysis of CF and CA serum titers showed a significant and marked correlation (Figure 1).

Rapid Card Agglutination Test

The most recent serologic test for the diagnosis of anaplasmosis has been the rapid card agglutination test (CT) developed by Amerault and Roby (3, 4). The CT has several advantages, and is now an officially recognized test for anaplasmosis, along with the CF test. The *Anaplasma* antigen is prepared from infected erythrocytes. It is purified by separating the erythrocytic stroma and antigenic particles by passage through a French pressure cell. The purified antigen is stained for ease in reading. As with the CA test it is very simple to set up and to read. Care is needed, however, to ensure best results. The CT was primarily developed to be a field test that could be conducted at the ranch using non-inactivated plasma. In the laboratory, the test can be done on either non-inactivated serum or plasma.

Blood samples for field testing are obtained from the tail vein in a small plastic bulb containing a measured quantity of heparin. After shaking to ensure thorough mixing with heparin, the samples are

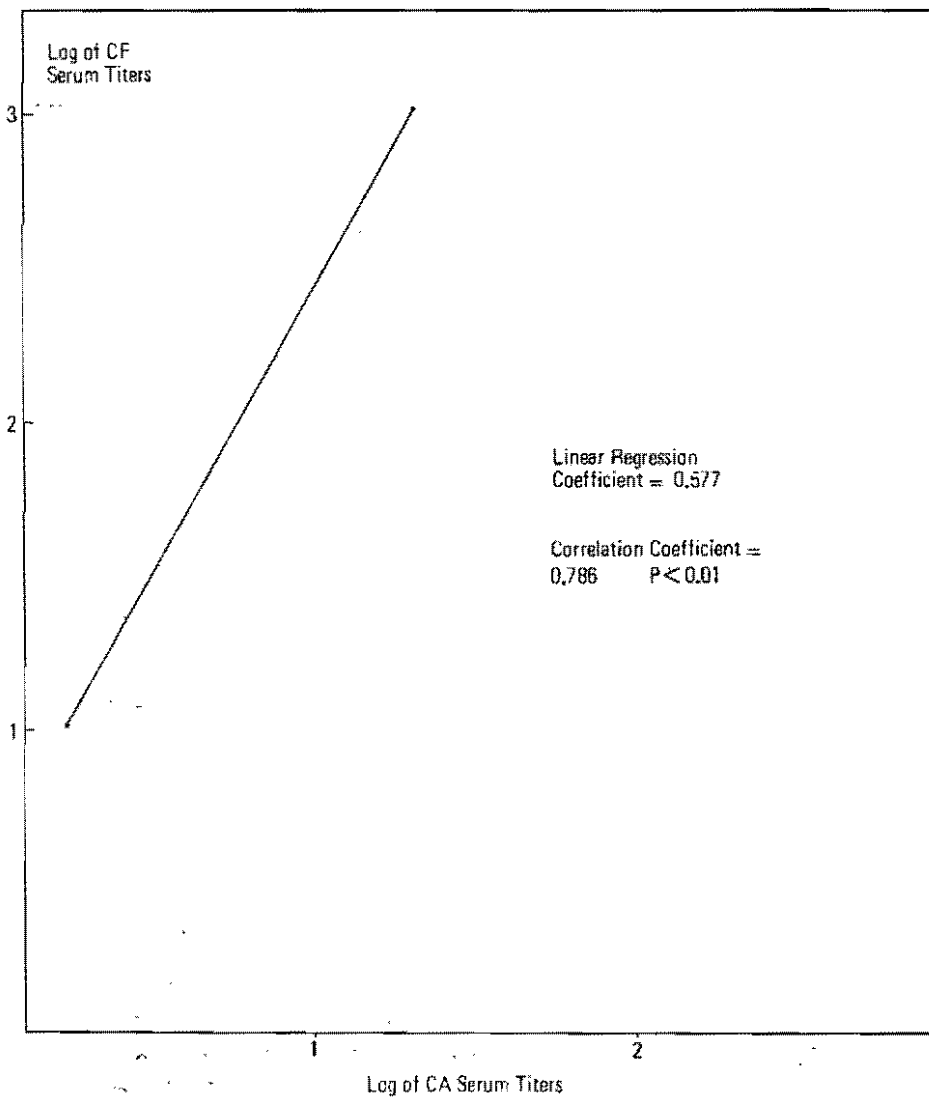


Fig. 1. Correlation of capillary-tube agglutination and complement-fixation serum titers in known anaplasmosis-infected cattle based on 21 serum titrations.

centrifuged four minutes at 2000 x G. in a small centrifuge operated from a car battery.

A bovine serum factor (BSF) is supplied with the test kit. This substance is reconstituted with 3.2 ml distilled water. Exactly two drops (0.03 ml) of the BSF are dispensed onto each 18 mm circle test area. Using a marked capillary tube, 0.03 ml of cell free plasma is placed in the test circle. One drop of antigen (0.015 ml) is added

Table 4. Serologic examination of b69 cattle comparing the CF and card test.

Complement Fixation		Card Test	% Agreement
Pos.	322 (68.7%)	389 (82.9%)	96.8%
Susp.	80 (17.1%)		
Neg.	67 (14.3%)	80 (17.1%)	83.8%
Overall Agreement			94.8%

to the plasma and BSF. The three substances are mixed, using a toothpick or other flat object, and then the card is placed on a rotating plate, under a humidifying cover for 4 minutes, being rotated at the rate of 100 RPM. Following rotation, the card is tilted once (to and fro) and immediately read macroscopically. A positive shows characteristic clumping; a negative does not show the clumping. Caution should be taken not to delay reading, since drying and prolonged manual mixing may well result in a non-specific reaction.

Comparisons between the card test and complement-fixation tests, for the most part, show good agreement.

Two studies have recently been made in areas of high anaplasmosis incidence. The first such study was conducted on 469 cattle in South Texas. The CT was done on plasma at the ranch. Serum was collected from duplicate samples and tested using the CF in the laboratory. The serum was not phenolized. The second test was done on sera from 164 cattle in Guyana. The CT and CF were conducted on serum.

The results are tabulated in Tables 4, 5, 6, and 7. The overall agreement of the CT using plasma and CF test on serum was 94.8% (Table 4). In this instance, the CF test appeared to be slightly more sensitive (Table 5). This may have been due to the nature of infection in this herd. There were many new cases of anaplasmosis as seen in studies of packed cell volume and blood smears. It is generally recognized that the CF test becomes positive more rapidly than the agglutination test (both the CT and CA).

Table 5. An analysis of differences occurring between the CF and card tests.

Total No. of Samples Not Agreeing	CF Positive CT Negative	CF Suspicious CT Negative	CF Negative CT Positive
34 (7.2%)	15 (44.1%)	9 (26.5%)	10 (29.4%)

Table 6. Serologic comparison of 164 cattle comparing the CF and the card test (serum).

Complement Fixation	Card Test	% Agreement
Pos. & Suspicious 180 (66%)	117 (71%)	92.3%
Negative 56 (34%)	47 (29%)	83.9%
TOTAL 164	164	89.7%

In the second trial on 164 cattle in Guyana, the overall agreement was 89.7% (Table 6). A breakdown of disagreement in Table 7 indicates the CT is more sensitive than the CF. This may reflect the overall stage or length of infection in tested animals. If this is the case, these results would confirm the view that the CF is more sensitive in recent and acute infections, with the CT being better in detecting long-standing infections.

Table 7. An analysis of differences occurring between the CF and card tests (serum)

Total No. of Samples Not Agreeing	CF Positive & CF Suspicious CT Negative	CF Negative CT Positive
24 (15%)	8 (33%)	16 (67%)

Summary

Reliable tests are available to diagnose both acute and chronic anaplasmosis. A high degree of correlation and agreement occurs between the complement-fixation (CF) and the capillary tube agglutination test, and between the CF and the rapid card test (CT). Both the CF and CT are recognized as official tests for interstate movement of cattle where regulations require a preshipment negative test.

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METHODS OF IMMUNOPROPHYLAXIS AGAINST BOVINE ANAPLASMOSIS WITH EMPHASIS ON THE USE OF THE ATTENUATED *ANAPLASMA MARGINALE* VACCINE

*Miodrag Ristic * and C. A. Carson **

Introduction

A common feature of hemotropic diseases is the continuous presence of the etiologic agent in the blood circulatory system. All hemotropic diseases are transmitted by arthropod vectors. Certain of these (anaplasmosis and babesiosis) cause devastating losses to the livestock industry and others (malaria and trypanosomiasis) are of great importance to human health.

One of the major obstacles to the development of methods for immunoprophylaxis against hemotropic diseases has been the lack of techniques for *in vitro* propagation of the causative agents (plasmodium, anaplasma, babesia), or the availability of similar systems for laboratory production of arthropod-associated "prototype" antigens (trypanosomes). A recent accomplishment in this direction has been the development of cell culture methods for *Theileria parva*, the causative agent of East Coast fever. While this step is considered an important break-through in providing incentive for more optimistic future endeavors in the entire field, the accomplishment must not be viewed as an indication that this technique can be directly applied to other hemotropic agents. *Theileria parva* actively invades cellular elements of both the erythrocytic and lymphocytic series, the latter being adapted to *in vitro* propagation. *Anaplasma*, *Babesia*, and *Plasmodium* spp. are considered to primarily affect the more inert circulating erythrocyte which, rather than offering replication ability in cell culture, continues its *in vitro* destiny of degeneration.

Anaplasmosis is a world-wide tick-borne disease of cattle and some wild ruminants caused by the rickettsia *Anaplasma marginale*. The organism is extremely host specific and the mature erythrocyte constitutes the only cell known to support growth and development

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of the agent in an infected animal. "Premunition", which consists of infecting, and subsequently treating an animal, to establish a carrier infection, is the oldest preventive method. It is, however, hazardous, time consuming, and costly.

Research toward the development of an *A. marginale* immunogen may utilize the blood of an animal in the acute phase of infection or infected arthropod vectors (tick) as possible sources of the antigen. In view of the limited knowledge regarding development of the organism in the tick, the latter source must presently be considered as theoretical. A degree of transient protection has been induced by inoculation of susceptible cattle with inactivated *Anaplasma* derived from the blood of infected animals and introduced in oil adjuvant. The method, however, caused development of isoimmunity in vaccinated animals followed by often fatal erythroblastosis in calves of vaccinated dams. Moreover, the procedure is laborious and expensive since the 2 initial doses of vaccine must be followed by yearly revaccination to maintain a level of protection.

In view of the aforementioned information, as well as other data, our laboratory developed and attenuated *A. marginale* vaccine by adapting *A. marginale* to growth in sheep, an atypical host. After 8 years of research, a strain of *Anaplasma* was derived which was safe for susceptible cattle yet sufficiently immunogenic to protect inoculated animals against laboratory and field challenge with the virulent organism. Although not original in concept, the method signifies the first successful adaptation of an erythrocytic parasite for the solution of a disease problem.

Biologic Properties of *Anaplasma marginale*

A. The Organism and Related Species

At the beginning of this century Theiler (1) described a small punctiform body which occurred in the erythrocytes of African cattle suffering from an acute infectious anemia. On the basis of staining characteristics, the author concluded that the organism lacked cytoplasm and used the term "anaplasma" to indicate this property and the term "marginale" to indicate the peripheral location of the organism within erythrocytes.

In the 8th edition of Bergey's Manual, *A. marginale* is the representative species of the genus *Anaplasma*, family Anaplasmataceae, order Rickettsiales, Ristic and Kreier (2), (Fig. 1). The organism (initial body) was shown to enter the erythrocyte by invagination of the cytoplasmic membrane with subsequent formation of a vacuole (3). Thereafter the initial body multiplies by binary

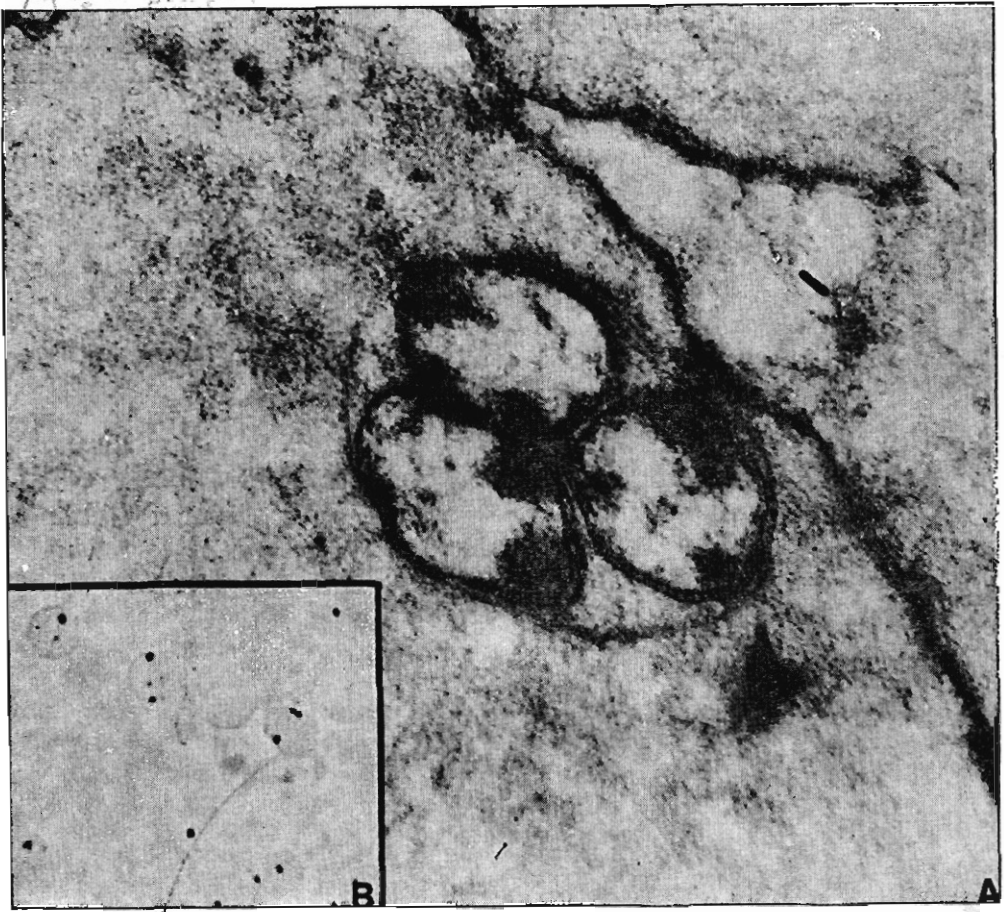


Fig. 1. (A) Ultrathin sections of an erythrocyte containing marginal anaplasma body with 3 initial bodies. X 118,000. (B) Blood film from a cow in the acute phase of anaplasmosis stained by Giemsa method. X 1,400.

fission and forms an inclusion body which consists generally of 4 to 8 initial bodies (4) (Fig. 2). Inclusion bodies are numerous during the acute phase of the infection, however, low-level infections persist for several years thereafter. Of the 3 *Anaplasma* species, *A. marginale* is the most pathogenic for cattle. *Anaplasma centrale* causes a relatively "mild" form of bovine anaplasmosis in Africa and *Anaplasma ovis* is the cause of ovine and caprine anaplasmosis.

Paranaplasma caudatum (genus *Paranaplasma*) Kreier and Ristic (5, 6, 7) was initially found in Oregon cattle in a mixed infection with *A. marginale*. Inclusion bodies of *P. caudatum* have appendages usually in the form of a tapering tail, a loop or a ring, demonstrated

only by use of special techniques. A recent study by Carson et al (8). in our laboratory showed that manifestation of *P. caudatum* appendages is a function of bovine host erythrocytes and does not occur in infected deer erythrocytes.

The various anaplasma organisms have at least one species specific antigen and they are sensitive to the tetracycline group of antibiotics. Anaplasmas morphologically resemble other hemotropic rickettsiae, i.e. *Haemobartonella* and *Eperythrozoon* and share common antigens with these agents (9). Ristic (10, 11) has compiled general reviews on anaplasmosis, the parasite itself, and the host response.

Arthropod Vectors and Epizootiology

Experimentally, at least 20 tick species have been shown to transmit anaplasmosis although field evidence indicating the tick as the principal disease vector is lacking (11). Histochemical staining, fluorescent antibody methods, and electron microscopy have been used for identification of the organism in the tissues of vector ticks. The

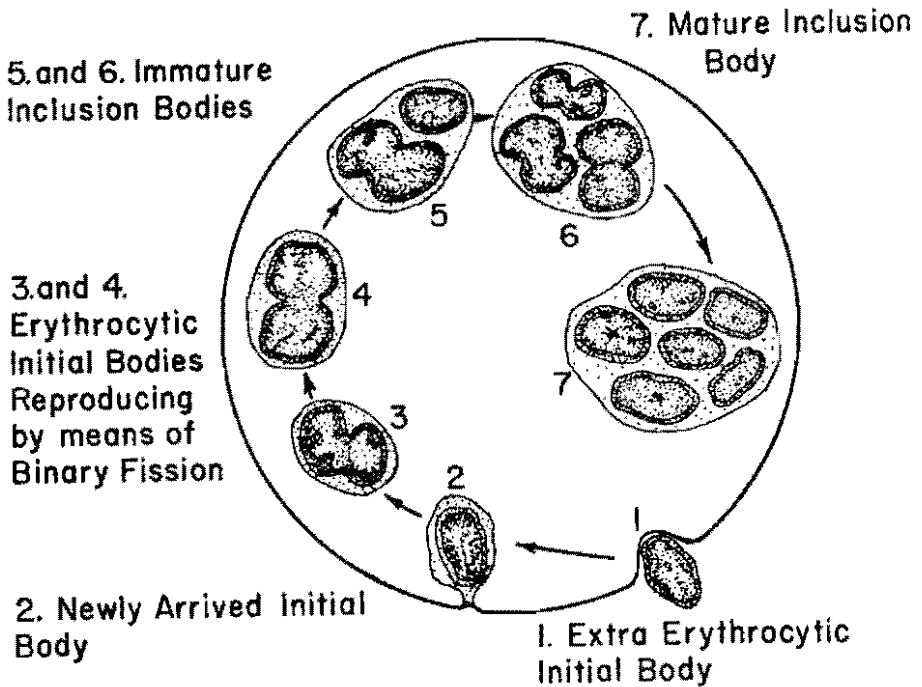


Fig. 2. Proposed developmental cycle of *Anaplasma marginale* based on static evidence obtained from electron microscopic studies.

organism was demonstrated in the gut contents and in the Malphigian tubules of engorged *Dermacentor andersoni* ticks, however, little is known regarding its developmental life cycle (12,13). Experimental and epizootiological evidence incriminates horse flies (*Tabanus* spp.) as the most significant insect vector of anaplasmosis. Transmission by flies is affected by the direct transfer of blood from infected to susceptible cattle and must take place within a few minutes after feeding on an infected animal. Experimental evidence of transmission was also produced with stable flies (*Stomoxys*), deer flies (*Chrysops*), horse flies (*Siphona*), and mosquitoes of the genus *Psorophora* (11).

In addition to carrier cattle, wild deer have been shown to play an important role in the epizootiology of anaplasmosis. Extensive studies in California indicate that *Anaplasma* may survive in nature in the absence of cattle through deer-to-deer transfer via appropriate vectors. Transmission of infection from deer to cattle has also been demonstrated by several investigators (11).

Intraerythrocytic Behavior, Transfer, and Circulatory Clearance of Infected Erythrocytes

According to Moulder (14), a successful intracellular parasite must avoid its demise by regulating its growth rate so that demands on the host do not exceed tolerable limits. *Anaplasma* meets this criterion since it enters host erythrocytes and replicates without causing mortal injury of the host cell. It also appears that the kinetics of anaplasma initial body movement between erythrocytes occurs in a manner which avoids irreparable damage of the host cell membrane. Incubation of *A. marginale* in an *in vitro* system showed that the decrease of observable marginal bodies equalled twice the degree of hemolysis, indicating that the organism may leave the erythrocyte without concomitant host cell lysis (15). The organism may be able to act on the host cell membrane by using a proteinase-like system similar to that related to some Chlamydiae, providing for organismal release without drastic disturbance of the cell membrane (16). Since the organism is rarely observed extracellularly, the transit may occur through intercellular tissue bridges. The consequence of organismal interaction with erythrocytic membrane is indicated by a decrease in the concentration of phospholipids (17) in erythrocytic stroma and a high serum sialic acid concentration (18). The removal of the organism from the circulation takes place by phagocytosis of entire infected erythrocytes. Phagocytosis of apparently noninfected erythrocytes, as frequently observed, may be caused by stimulation of an autoimmune response due to erythrocytic membrane alterations by *Anaplasma*.

Glycine incorporation into protein by *Anaplasma* infected erythrocytes *in vitro* has been the only available metabolic information associated with *Anaplasma* infection of the host cell (19). Recently Simpson et al. (20) indicated the presence of a food vacuolelike structure inside an erythrocytic "matrix" harboring initial bodies. Concurrent studies in our laboratories confirmed these findings (Fig. 3). Thus a partial analogy of food intake among *Plasmodium*, *Babesia*, and *Anaplasma* spp. may exist. Unlike *Plasmodium* and *Babesia* however, the phagotrophy leading to formation of the food vacuole in *Anaplasma* spp. is extraparasitic and the underlying mechanism not understood.

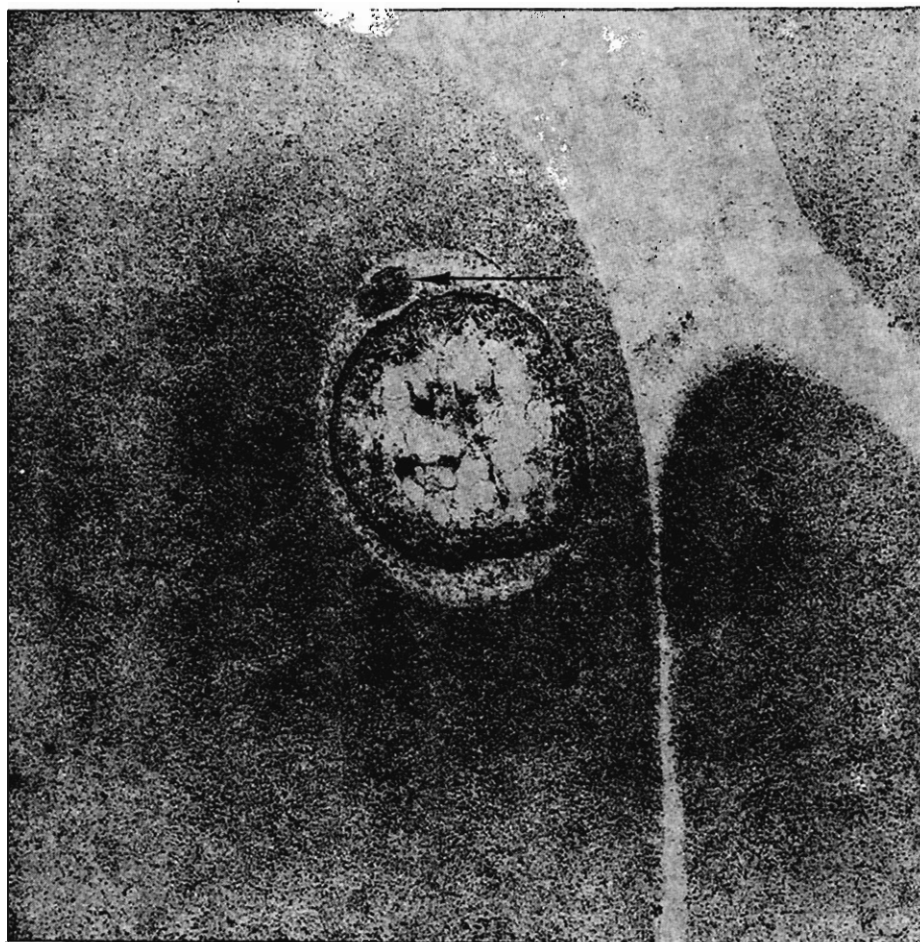


Fig. 3. Ultrathin section of an erythrocyte containing marginal *Anaplasma* body with 1 initial body. Note the presence of a food vacuole in the erythrocytic vacuole surrounding the organism (Arrow) x 122,000 (approximately).

Antigenic and Serologic Studies of *A. marginale*

As a prelude to the development of immunoprophylactic measures, a complete understanding of the antigen structure of *Anaplasma* becomes necessary. Subsequent use of information related to the organism's antigenic profile can then support immunogenic studies based on biochemically defined constituents.

Intraerythrocytic Antigens

Soluble HCL and protamine sulfate (PC) precipitated *A. marginale* antigens have been found to be lipoproteins. The antigens were reacted with antibody in the micro agar gel precipitation test (21, 22). Clinical resemblance of the PS antigen to a similar preparation made from *Anaplasma*-free erythrocytes incriminated it as a possible antigen responsible for the autoimmune state proposed in anaplasmosis. A soluble proteinaceous *A. marginale* antigen has been derived from lysed erythrocytes and its reactivity studied in agar gel diffusion (23).

Various particulate antigens for diagnostic tests have been prepared from infected erythrocytes (24, 25, 26). The active component of the complement fixation (CF) antigen was found to be a lipoprotein (24). Capillary tube agglutination (CA) antigen consists of structures resembling initial *Anaplasma* bodies which reacted specifically with sera from infected animals (25).

No antigenic differences were found between two virulent *A. marginale* isolates and the attenuated *A. marginale* when soluble antigens and specific antisera were reacted in agar gel and immunoelectrophoresis tests (27).

Serum Antigens

Serum, as well as lysed erythrocyte preparations from animals in the acute phase of anaplasmosis, have been found to contain soluble antigens of *A. marginale* (28). Cyclic emission of soluble parasite antigen was indicated by fluctuation of test results. The presence of detectable intraerythrocytic *A. marginale* bodies did not always coincide with the presence of soluble antigens in serum. It also appeared that a soluble component of the CF antigen and the exo-antigen found in serum of infected cattle were identical.

Serology and Kinetics of the Humoral Response

Serum protein changes occurring during anaplasmosis, particularly with respect to globulin, have been studied extensively (29, 30). Prior to the appearance of *A. marginale* bodies, the concentration of all serum proteins decreased. As the number of parasitized erythrocytes diminished the albumin-globulin ratio decreased. It was determined

that early CF antibody arising in response to experimental inoculation of *A. marginale* consisted exclusively of gamma M (19 S) globulin, but within 4 to 5 days this was augmented by an electrophoretically fast gamma G globulin (6.2 S). Agglutinating antibodies moved from the gamma M to the gamma G globulins much later in the disease (30).

The serologic relationship of *A. marginale* and *A. centrale* have been compared using the CF and CA tests. Significantly higher titers were obtained with homologous antigen (31). The indirect Coon's test also showed quantitative differences in antibody titers produced in response to infections with the above agents (32).

Persistence of the Organism in Immunologically Hostile Hosts

It is apparent from our current knowledge of anaplasmosis that an affected animal is fully capable of developing humoral and cell mediated immune responses to the etiologic agent. While induction of an immune response is essential for the survival of the host, it also provides for continuous survival and transmission of the parasite. This state of biological host-parasite balance has been described as a "tolerant symbiosis" which allows the parasite to persist and, in exchange, the host develops lasting protection against homologous organisms in the environment (33). Obviously there are different mechanisms which the parasite may use to avoid destruction by the immunologically hostile host. The nature of these escape mechanisms in plasmodia, trypanosomes, babesia, and certain other species was the subject of a recent symposium (34). We should briefly discuss possible means whereby *Anaplasma* might avoid destruction in an immune host.

Without evidence of intravascular hemolysis, *Anaplasma* appear to be strict intracellular obligate parasites. Under the circumstance, the organism is well protected against direct antibody affects. Antibodies arising in response to *Anaplasma* infection are of 2 types, specific and nonspecific. The latter, characterized as autohemagglutinins and opsonins, were shown to react with nonparasite antigenic determinants such as trypsin-treated and intact erythrocytes of *Anaplasma*-free animals (11). In addition, some of these immunoglobulins were shown to be anti-nuclear (anti-DNP); anti-cardiolipin (anti-Wasserman antigen), and anti-human gammaglobulin reminiscent of the rheumatoid factors (35). Greenwood (36) proposed that the appearance of such nonspecific antibodies suggests their synthesis occurs in a disorganized way. It is thus possible that the nonspecific immune response may benefit the parasite by "swamping" a specific immunological response aimed at its destruction.

Evidence for antigenic variation of *Anaplasma* is not available. Clinical and hematologic observations, however, indicate that the primary parasitemia may be followed by milder recrudescence. If these relapses are triggered by antigenic dissociation of the organism, this could be a primary factor whereby the *Anaplasma* evades the host's lethal immune effect.

Free serum antigens were shown to be *Anaplasma* specific thus, antigen-antibody complexes are expected to occur. Such complexes may induce immunologic injuries to the host while serving as a protective shield for the parasite.

Immunogens of *Anaplasma*

Naturally Occurring *A. marginale* and *A. centrale*

Early use of field isolates of *Anaplasma* for premunization consisted of injecting blood from known carriers into susceptible cattle (31). This procedure presented a certain risk since it was often necessary to control the initial infection with drugs so subsequent recovery and development of the carrier state could occur. A method of premunization using a small inocula to induce protection has also been reported (38) but later results indicated that severe disease and mortality can result from even very small doses of *A. marginale* (39).

Prior infection with *A. centrale* did not prevent infection with *A. marginale* but reduced the severity of the superimposed diseases. On this basis Theiler (1) developed the method of *A. centrale* vaccination which is still used in several countries. Variable resistance to *A. centrale* was later described (40). Furthermore, it was observed in Australia that cattle inoculated with *A. centrale* (South African) strain developed severe symptoms of the disease. *Anaplasma marginale* and *A. centrale* have more recently been compared on a clinical, hematologic, and serologic basis (31, 41). Heterologous challenge in all cases produced a definite hematologic reaction but *A. centrale* usually reduced the severity of a subsequent *A. marginale* challenge.

Inactivated *A. marginale* of Bovine and Ovine Origin

Use of a vaccine containing killed *Anaplasma* organisms has been described in Africa (42) and the U.S. (43). The U.S. vaccine presently used commercially, "Anaplaz",* was prepared from blood of infected animals collected at the peak of parasitemia. Blood cells were washed,

* Anaplaz. Fort Dodge Laboratories, Fort Dodge, Iowa.

lysed, and lyophilized. The desiccated material was reconstituted with an oil adjuvant. Two doses of the vaccine were given subcutaneously at 4 to 19-week intervals. A degree of protection from the clinical signs of anaplasmosis has been reported to be afforded 2 weeks after the second injection. Vaccinated animals became carriers after field challenge. An annual booster injection is also recommended.

A preparation similar to Anaplaz was developed in our laboratory using ovine erythrocytes infected with the attenuated *A. marginale*. The Anaplaz adjuvant has been used to reconstitute the lyophilized material and the aforementioned vaccination regimen followed. A degree of resistance to development of clinical signs of anaplasmosis has similarly been detected after challenge with virulent *A. marginale*.

Laboratory Attenuated *A. marginale*

Research toward development of the attenuated anaplasmosis vaccine started at the University of Illinois in 1959 with the following objectives in mind: 1) The strain should be *A. marginale*; 2) the attenuated organism should be sufficiently immunogenic to confer complete protection against challenge with the virulent strain; 3) the attenuated organism should not revert to virulence when transmitted by means of ticks or subinoculation of blood from vaccinated to susceptible cattle; and 4) the vaccine should be produced in a non bovine host due to the hazards of transmitting other bovine infectious agents in the process of vaccination.

1. **The development of the strain.**- The *A. marginale* isolate was a pool sample collected from naturally-infected cattle in various sections of Florida and was collectively termed the Florida *Anaplasma** isolate. Detailed description of the development of the vaccine strain is reported elsewhere (44). The basic methodology used for attenuation of the Florida isolate has been: 1) induction of an apparently accelerated rate of mutation of the organism by exposure to irradiation; 2) selection of an avirulent *A. marginale* strain by serial passage of irradiated organisms in splenectomized deer (2 passages) and sheep (138 passages). Lots of sheep blood derived seed material of established immunogenicity, growth pattern, and safety were preserved by storage in liquid nitrogen. For vaccine production, the seed material is reactivated in splenectomized sheep, using 4 to 5 passages at 7- to 9-day intervals. The vaccine is dispensed in liquid nitrogen and inoculated intramuscularly in 1 to 2 ml. doses. The prepatent period in inoculated animals varies between 4 and 6 weeks after which the

** Isolated in 1955 by Dr. D.A. Sanders, Department of Veterinary Science, University of Florida, Gainesville, Fla.

organism may be detected in approximately 0.5 to 8% of the peripheral blood erythrocytes. A slight hematocrit decrease, usually not exceeding 5 to 10% of the preinoculation value, generally occurs. These manifestations are transitory and are in evidence for 1 to 2 weeks. Inoculated animals are not clinically affected.

2. **Effects of adaptation to an atypical host.**- Adaptation of the organism to sheep resulted in the loss of certain traits apparently not essential for immunogenicity and intracellular existence of the organism. The loss of virulence is the principal and the most important of these.

The incubation period of the attenuated agent does not differ greatly from that of the virulent organism, thus the speed of growth and replication does not seem to be a factor in altered virulence. Attenuation appears to be a stable property as indicated by failure of the organism to revert to virulence after several consecutive passages (5 to 12) in highly susceptible mature cattle, pregnant cattle, and splenectomized calves. (45) The mechanism of altered virulence probably is biochemically based and arises from the organismal dependency on the new intracellular habitat.

The attenuated agent has apparently also lost the ability to be transmitted by ticks. An extensive study with *Dermacentor andersoni* (46) showed that the vaccine strain cannot be transmitted by natural tick feeding or inoculation of cattle with various tick tissue homogenates. These findings were further substantiated by the results obtained from examination of tick tissues by means of the fluorescent antibody method and electron microscopy. Conversely, *Anaplasma* was found in tissues of ticks fed on calves inoculated with the field strain of the organism and the infection was then transmitted to cattle using these ticks and their tissues. Although more information is needed to substantiate these findings, experience has shown that prolonged maintenance of hemotropic and other tick-borne agents by needle passage in natural hosts results in a loss of organismal propensity toward growth and development in the tick vector (47, 48).

Finally, the attenuated *A. marginale* is more easily destroyed by tetracyclines than is the virulent counterpart (45). For example, daily administration of 8.5 gm. tetracycline per pound of body weight to vaccinated cattle for 4 days completely destroyed the attenuated agent as contrasted to 10 mg/lb body weight for 7 days to completely destroy the virulent organism.

Immune Responses to Inactivated *A. marginale* Vaccines

Humoral and Cell-Mediated Responses

The commercial inactivated *A. marginale* vaccine called Anaplaz composed of infected bovine erythrocytes produced a variable and transient CF response (49). A study in our laboratory using both Anaplaz and ovine origin counterpart indicated that the CA response remains mildly positive for at least 6 weeks after the second vaccine dose. We further compared the cell-mediated immune response to inactivated *A. marginale* with that of virulent *A. marginale*. The leukocyte migration inhibition test (LMIT) and the lymphocyte transformation test (LTT) were used as *in vitro* correlates of cell mediated immunity (CMI). One group of cattle was vaccinated with 2 doses of Anaplaz administered 4 weeks apart. A second group of cattle received a lyophilized preparation of *A. marginale* produced by exposing ovine erythrocytes containing the attenuated *A. marginale* to an extraction process similar to that used for commercial preparation of Anaplaz. The immunogen was reconstituted in the Anaplaz oil adjuvant and administered as recommended for the commercial preparation. Both vaccines elicited a very low level and transient (1-3 weeks) LMIT response.

Lymphocyte transformation studies using (14C) labeled thymidine revealed a lack of correlation between test results and clinical protection. Stimulation indices after vaccination, defined as the ratio of incorporated activity in antigen stimulated cell cultures compared to control cultures, were in the order of 2 to 8 or higher compared to indices of 1.2 to 2.5 in leukocytes collected from cattle inoculated with replicating forms of *A. marginale*. Since cattle which have recovered from *A. marginale* infection are clinically protected against challenge by homologous strains of *Anaplasma*, there seems to be no direct correlation between stimulation indices and protection (Fig. 4).

Development of Isoimmunity and Delayed Cutaneous Hyper-sensitivity.

Neonatal isoerythrolysis has been associated with the use of the Anaplaz vaccine (50). Hemolytic inhibition tests have indicated the presence of bovine blood group antigens in Anaplaz (51). Blood group isoantibodies identified in the cows' sera and colostrum (in addition to neutral anti-J) were anti-A, anti-H, anti-F and anti-V. Their concentration in colostrum was 10 to 15 times greater than in serum (51). Our studies which compared inactivated *A. marginale* preparations containing bovine erythrocytic components with similar

preparations containing ovine erythrocytic stroma, demonstrated that isoantibodies to bovine erythrocytes developed only in response to inoculation with infected bovine erythrocytes. Intradermic skin testing with antigens prepared from normal bovine and ovine erythrocytes showed that cattle which had been vaccinated with Anaplaz of bovine origin were skin-test positive to both bovine and ovine erythrocytic components while those which had received the ovine origin anaplasma reacted only to ovine origin erythrocytes. The skin reactions appeared prior to 24 hours indicating an immediate or Arthus-type response involving humoral antibody. This response increased over the 72-hour surveillance period indicating a delayed cutaneous hypersensitivity limb of the response.

Protection and Immunologic Injury Following Challenge.

Studies in our laboratory showed that cattle vaccinated with inactivated *A. marginale* of either bovine or ovine origin developed on challenge a low level parasitemia with a greatly reduced hematocrit (Fig. 4). The severe anemic syndrome which developed following challenge may be related to an autohemolytic mechanism in view of the fact that abnormal erythrocyte components are included in the vaccinal inocula and have definite immunogenic properties. Subsequent infection of host erythrocytes changes membranous constituents which the host may not distinguish from the original vaccinal components. This situation may lead to an accelerated autoimmune antierythrocytic host response.

Immune Responses to the Attenuated *A. marginale*

A. Hematology and Serology.

Hematologic responses in adult cattle to vaccination with the attenuated *A. marginale* have been extensively studied in our laboratory. This agent produces a mild infection with a concomitant low parasitemia and a generally imperceptible hematocrit variation at approximately 4 to 5 weeks after inoculation. Serologic responses measured by the CA or CF tests are detected between 3 and 4 weeks post-vaccination. These serologic responses persist for 2 to 3 years in the absence of reinfection (45).

In vitro Measurement of Cell-Mediated Immunity after Vaccination and Challenge.

After vaccination of susceptible adult cattle with attenuated *A. marginale*, the LMIT becomes positive after approximately 2 to 4 weeks and either remains at a moderately high level or fluctuates between prevaccination levels and positive response values (Fig. 5). Cattle inoculated with virulent *A. marginale* developed positive LMIT values

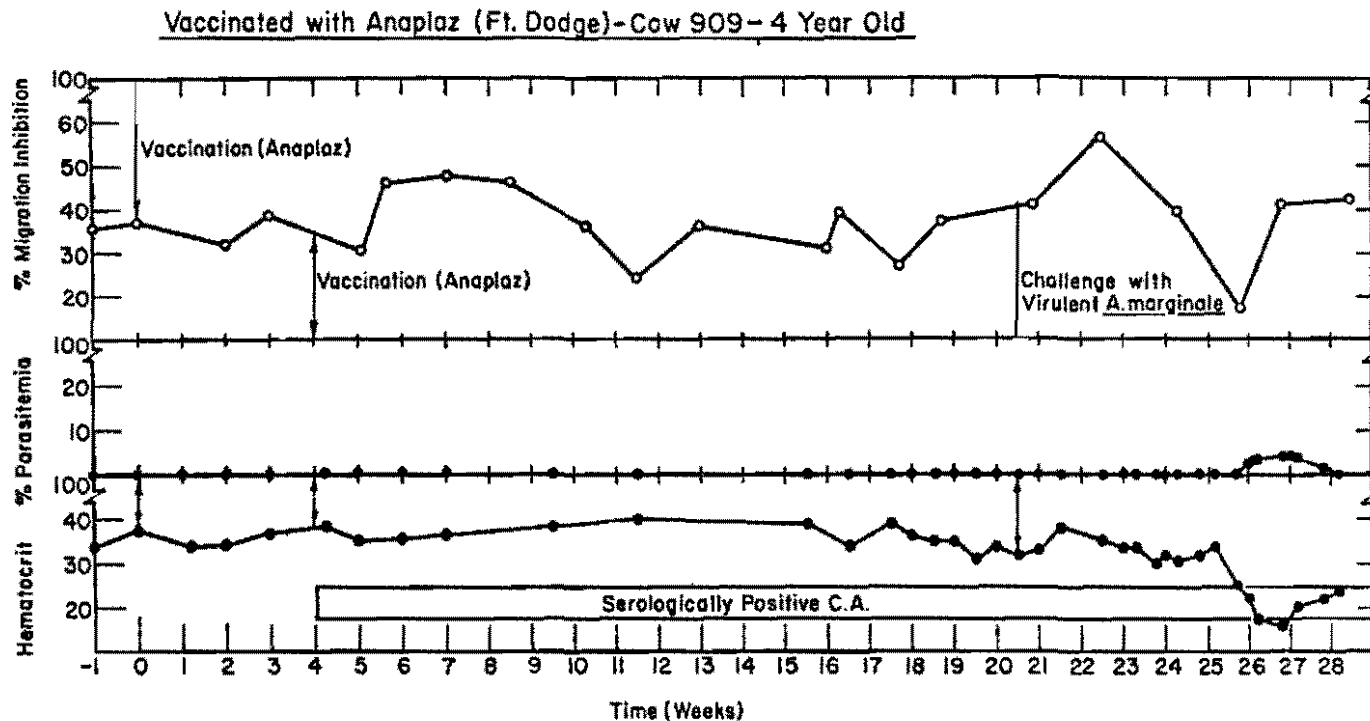


Fig. 4. Leukocyte migration inhibition, parasitemia, hematocrit, and capillary tube agglutination results for cow 909 vaccinated with Anaplaz and challenged with virulent *A. marginale* approximately 17 weeks after the second vaccinal injection. Severe anemia in the presence of relatively nil parasitemia was observed.

Vaccinated with Attenuated *A. marginale* - Cow 914 - 4 Year Old

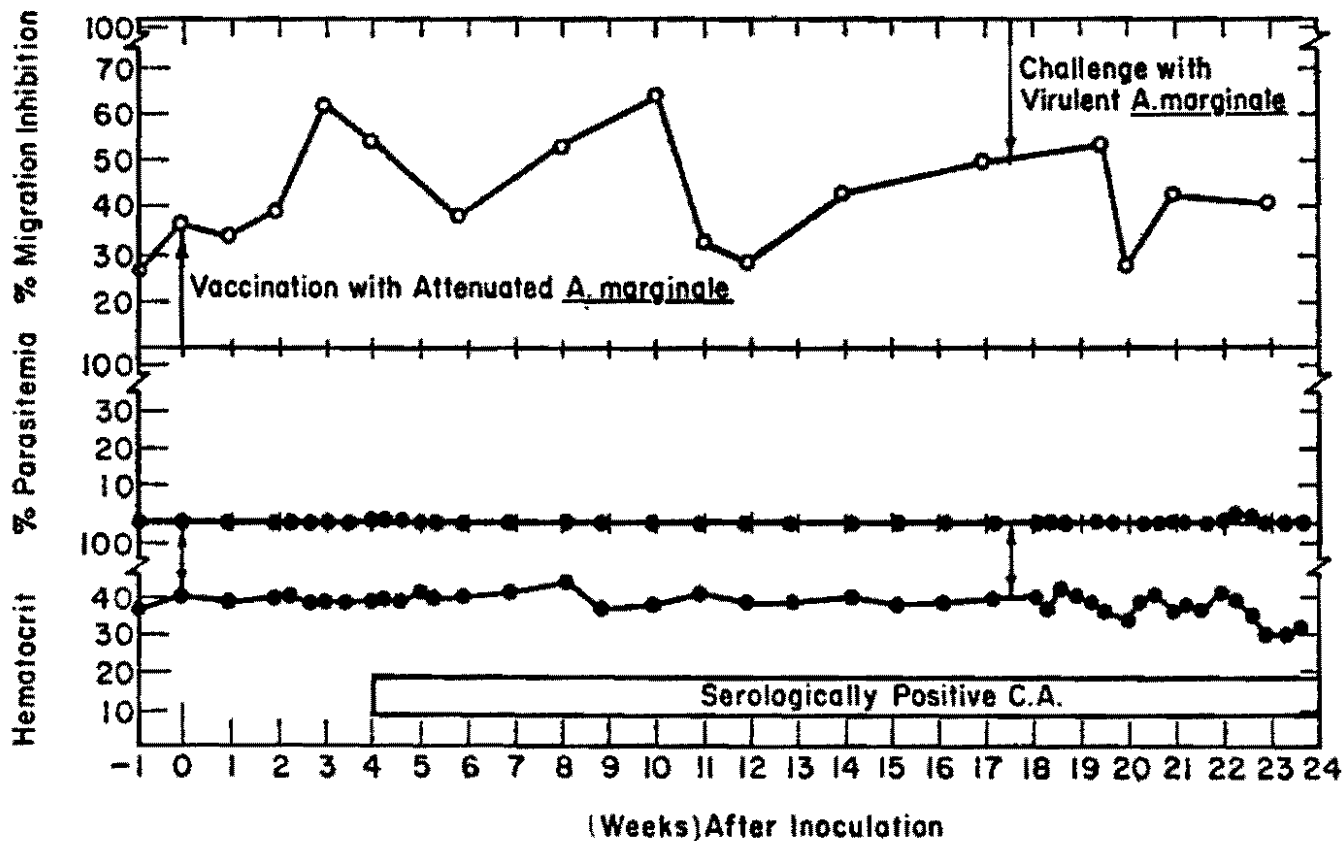


Fig. 5. Leukocyte migration inhibition, parasitemia, hematocrit, and capillary tube agglutination results for cow 914 injected with the attenuated *A. marginale* vaccine and challenged with virulent *A. marginale* approximately 17 weeks later. The animal demonstrated full clinical resistance.

either prior to or after the onset of parasitemia which generally occurred at 4 weeks post infection. The clinical severity of the disease depended on the time of appearance of parasitemia with reference to the LMIT. When the parasitemia preceded development of LMIT, the chance of eventual mortality was increased (Fig. 6). The specificity of the response has also been proven by use of test antigens containing normal erythrocyte stroma, CA antigen containing attenuated *Anaplasma*, CA antigen containing virulent *Anaplasma*, tuberculin (purified protein derivative) and nonspecific mitogens such as phytohemagglutinin and concanavalin A. Evidence supporting the concept that the test is largely dependent on T lymphocyte activity has also been determined using antithymocyte IgG labeled with ^{125}I and measurement of residual radioactive leukocyte populations before and after antigenic stimulation. Challenge of vaccinated cattle with virulent strains of *A. marginale* appears either to elicit a decreased measurable LMIT response followed by an elevated response (if LMIT values are high at the time of challenge) or an "anamnestic" elevation in the LMIT response.

The lymphocyte transformation test using ^{14}C labeled thymidine has also been related in our laboratory to vaccination of adult cattle with attenuated *A. marginale* or premunization with virulent *A. marginale*. Stimulation indices recorded after inoculation with either agent are comparable and range to the area of approximately 1.3 to 2.5.

Correlation between Cell-Mediated Immunity and Protection.

The level of LMIT responsiveness recorded after vaccination with attenuated *A. marginale* or premunization with virulent *A. marginale* seems to be directly proportional to the level of clinical protection induced against challenge with virulent *A. marginale*. Following challenge, either a low level or negligible parasitemia was recorded and the hematocrit either did not vary or was transiently reduced. There has been no evidence of clinical illness related to anaplasmosis after challenge (Figs. 6, 7).

Stimulation indices recorded in lymphocyte transformation test (LTT) of mixed leukocytes collected from vaccinated or premunized cattle were not related to the degree of clinical protection afforded by either *Anaplasma* inoculum. Much higher indices were elicited by the inactivated *Anaplasma* in oil adjuvant while these cattle were not clinically protected as were animals which received live *Anaplasma*.

Virulent *A. marginale* - Cow 835 - 3 Year Old

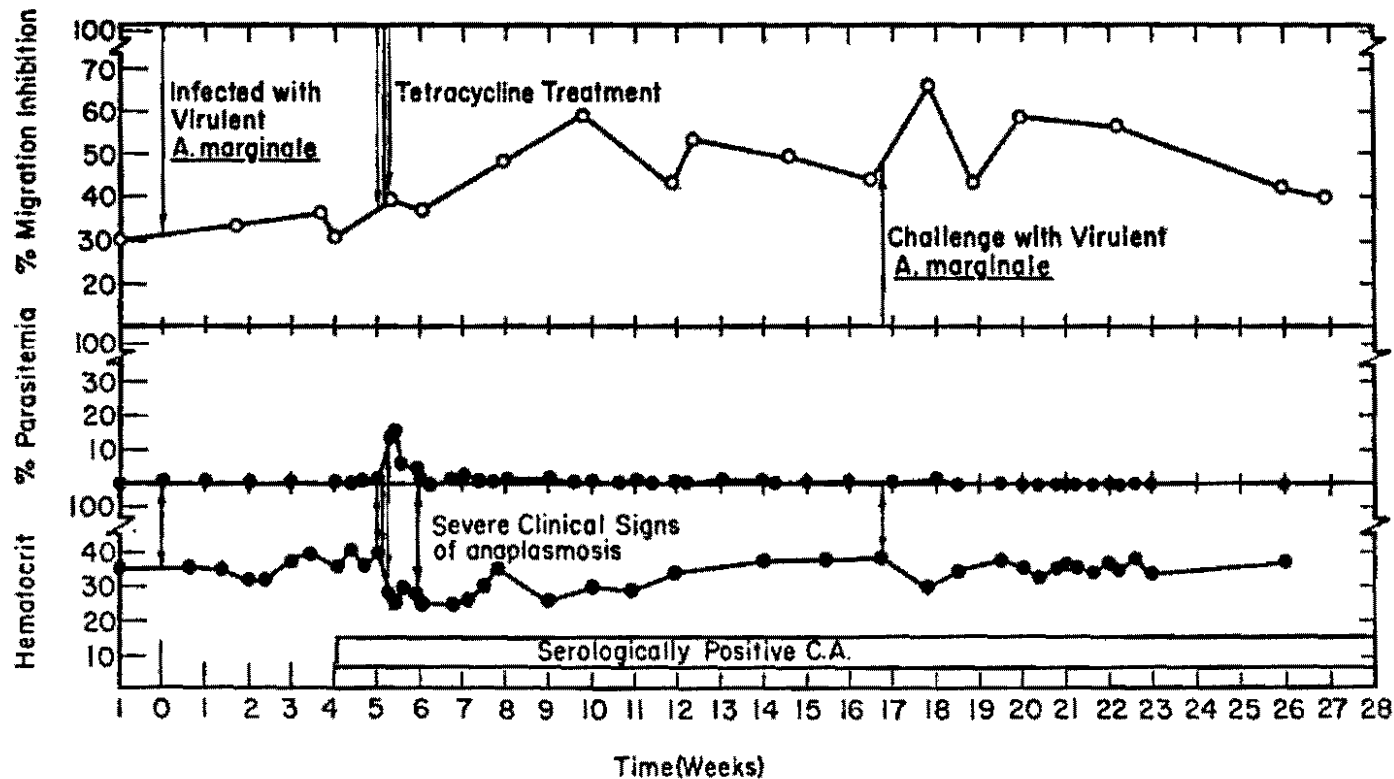


Fig. 6. Leukocyte migration inhibition, parasitemia, hematocrit, and capillary tube agglutination results for cow 835 injected with the virulent *A. marginale*. The animal developed severe signs of anaplasmosis and was treated 3 times with oxytetracycline at approximately 5 weeks after exposure. On challenge at 17 weeks after initial exposure, the animal demonstrated full clinical resistance. Note that this animal failed to develop appreciable LMIT response until recovered from the acute phase of the disease.

Attenuated *A. marginale* - Cow 923 - 3 Years Old.

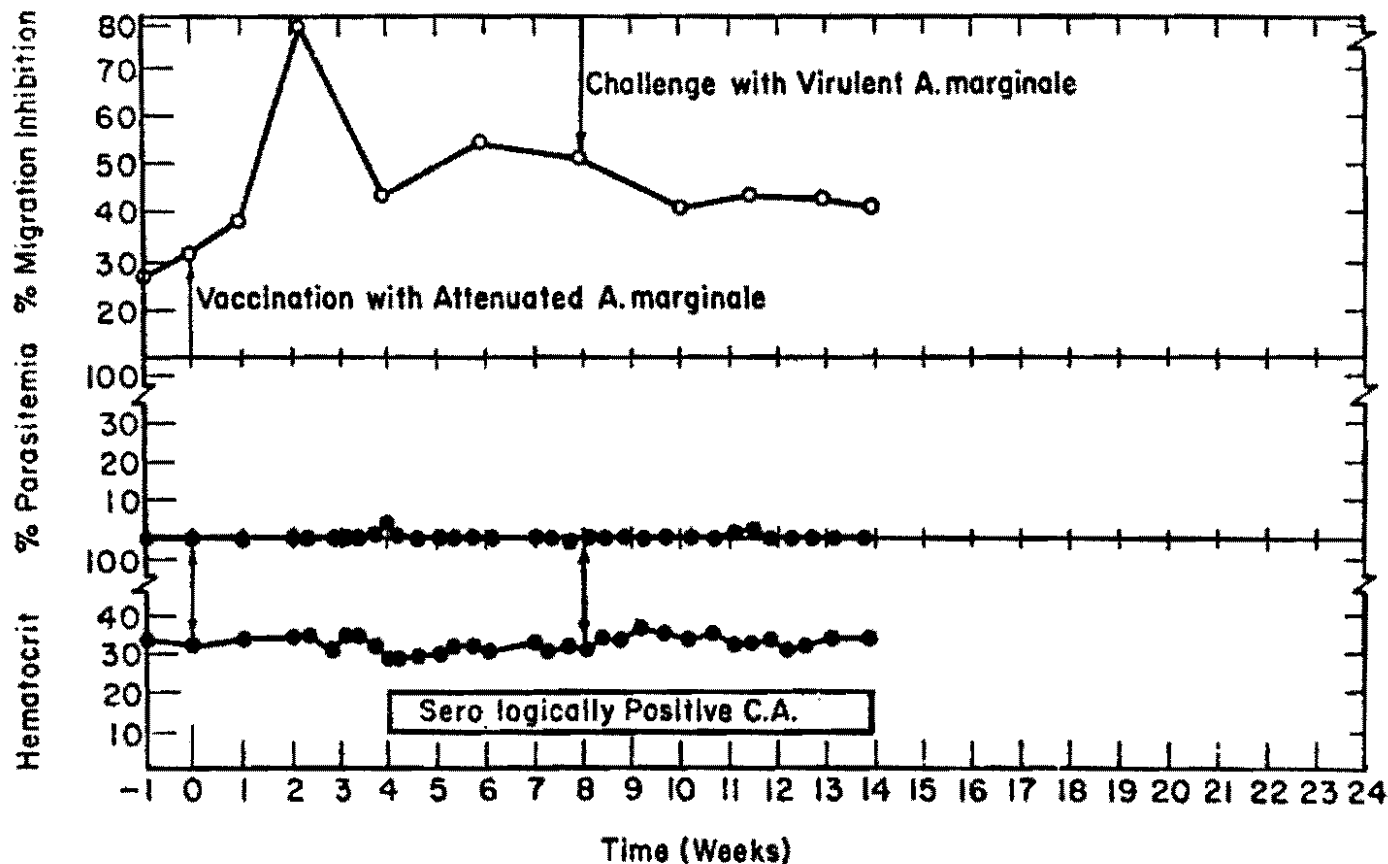


Fig. 7. Leukocyte migration inhibition, parasitemia, hematocrit, and capillary tube agglutination results for cow 923 injected with the attenuated *A. marginale* vaccine and challenged with virulent *A. marginale* 8 weeks later. The animal fully resisted the challenge. Note a prominent LMIT response with maximal values reached 2 to 3 weeks after vaccination.

The classical concept of protection in anaplasmosis has been that maintenance of protective immunity depended on maintenance of the carrier state. Recently, Roby et al. (52) reported that *Anaplasma* carriers which were freed from virulent *Anaplasma* by chemotherapy demonstrated considerable protection to challenge at 12 months after sterilization. To further examine this observation, we subjected two 4-year-old cows, which were previously vaccinated with the attenuated agent and subsequently resisted challenge of the virulent organism, to systemic therapy. The animals received 10 daily doses of oxytetracycline at 5 mg/lb administered intravenously. Subinoculation of 100 ml. of whole blood from these cows into 2 splenectomized calves failed to produce an infection in the recipient animals. Approximately 10 weeks after treatment, the cows were rechallenged with virulent *A. marginale*. A prompt LMIT response was noted after the challenge and the animals demonstrated full clinical resistance (Fig. 8).

Manifestations of LMIT, parasitemia, hematocrit and capillary tube agglutination test in a susceptible mature cow following challenge with virulent *A. marginale* are given (Fig. 9). It is apparent that maximal parasitemic and anemic crises preceded any appreciable response in the LMIT.

Vaccination Studies.

A series of laboratory and field experiment conducted in the United States, (44, 45, 53, 54) Perú, (55, 56, 57) Venezuela, (45,58) Colombia, (59, 60) and México (61, 62, 63) have shown that the immune response induced by this vaccine protects adult susceptible cattle against challenge with virulent endemic strains.

Most of the studies conducted in the U.S. were designed to test specificity, (53) safety (44, 45) and immunogenicity (44, 45, 54) of the vaccine using highly susceptible cattle (older animals, pure breeds, and pregnant animals) and challenging them with field isolates from various regions. Results showed that the single dose of the vaccine conferred solid protection to challenge with field isolates. As judged by the persistence of the serologic reaction in vaccinated animals, the protection is expected to last for at least 3 years. The largest experiment involved 2700 beef cattle of mixed breeds which were vaccinated on route from the U.S to Venezuela (45,58). On arrival, all animals were found to be serologically positive presumably as a result of vaccination. The losses in this herd were reduced from 9.5% in 1967, when nonvaccinated cattle were imported, to 2.5% when vaccinated cattle were imported. On arrival in Venezuela, the animals were exposed to a tropical climate and blood sucking arthropods

Vaccinated with Attenuated *A marginale* - Cow 845 en 4 Year Old

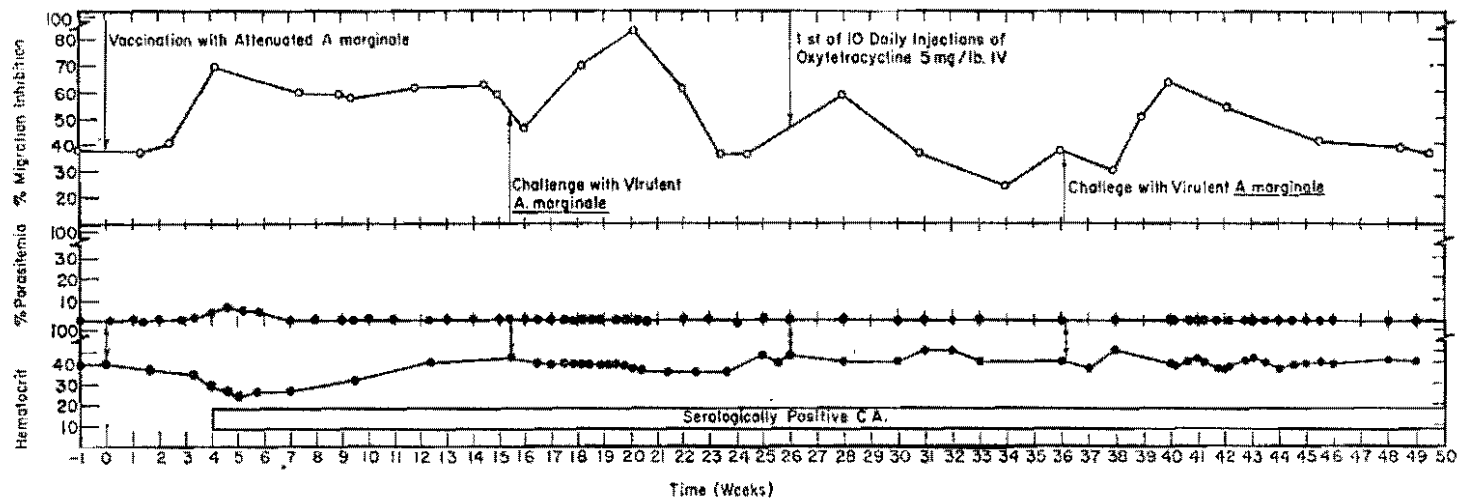


Fig. 8. Leukocyte migration inhibition, parasitemia, hematocrit, and capillary tube agglutination results for cow 845. This animal was initially injected with the attenuated vaccine, resisted challenge with virulent *A. marginale*, was treated with oxytetracycline to destroy the anaplasma and rechallenged with the virulent organism approximately 10 weeks after treatment. Note the rapid LMIT response following challenge. The animal fully resisted challenge.

known to be carriers of *Anaplasma* and other blood parasites. In spite of this unfavorable environment, no side effects, spontaneous reversion to virulence, or any other adverse effects were noted.

As an example of the efficacy of the vaccine, 4 controlled experiments conducted by veterinary government scientists in Perú (56) and México (61, 62, 63) will be cited. In Perú (56), 18 Brown Swiss cattle, 5 to 6 years old and free of *A. marginale*, were divided into 2 groups: vaccinated (13 cattle) and nonvaccinated (5 cattle). Forty eight days later, the 18 cattle were each challenged subcutaneously with 5 ml. of carrier blood containing a virulent Peruvian isolate of *A. marginale*. All control cattle developed typical signs of anaplasmosis

Unvaccinated Control - Cow 943 - 4 Year Old

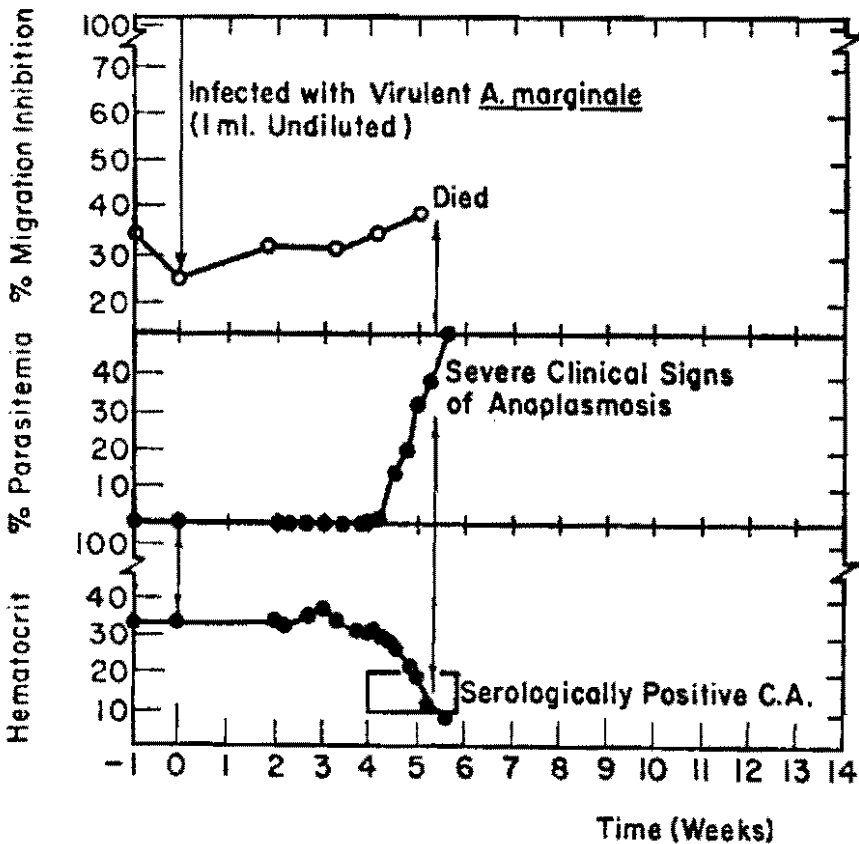


Fig. 9. Leukocyte migration inhibition, parasitemia, hematocrit and capillary tube agglutination results for cow 943 infected with virulent *A. marginale* but not treated. Note that the animal developed severe parasitemia and anemia but failed to develop a LMIT response before expiring 6 weeks after infection.

and died within 44 days after the challenge. Clinical signs of disease were not observed in any of the vaccinated animals following challenge inoculation.

In Mexico (61, 62), the efficacy of the attenuated *A. marginale* vaccine was evaluated in control experiments on 38 purebred dairy and beef cattle by scientists of the Instituto Nacional de Investigaciones Pecuarias, Mexico, D.F. In the laboratory experiment (Mexico City) vaccinated and control cattle (10 three-year-old Herefords in each group) were challenged with a virulent Mexican *Anaplasma* isolate 6 months after vaccination. The challenge dose, maintained in liquid nitrogen, had a standard potency sufficient to kill 80% of adult susceptible cattle. Challenge results showed 100% protection against mortality and 70% protection against morbidity in the vaccinated group as compared to 100% mortality in the control group. The short lasting morbidity in 30% of the vaccinates was followed by quick recovery.

In the field experiments (62), the cattle, which were raised in isolation units free of arthropods, consisted of 10 Brown Swiss calves (1 to 13 months of age) and 8 Holstein calves (5 to 7 months of age). They were paired by breed, age, and body weight, and allotted to 2 equal groups. Calves in 1 group were vaccinated, and 6 weeks after vaccinations calves in both groups were placed in the field where they were raised for approximately 1 year. Two Holstein and 3 Brown Swiss calves of the nonvaccinated group developed clinical anaplasmosis, and the remaining calves of this group had hematologic evidence of the disease during the 2 to 4 months after introduction to the field. The vaccinated group, which remained free of anaplasmosis, showed consistently greater weight gain than did the controls. Among the Holstein calves, the maximum weight difference in favor of the vaccinated group was 50 Kg/head at 5.5 months after field exposure, and among the Brown Swiss calves, the difference in weight gain in favor of vaccinated calves at the end of the 12-month period was between 11 and 30%.

Finally, a larger experiment recently completed in Mexico (63) involved movement of vaccinated and nonvaccinated animals from the northern tick-free portion of the country (Sonora) to the southern endemic region of Tabasco. There were 383 vaccinated and 42 nonvaccinated yearling cattle of mixed breeds. After 6 months residence in the endemic area there were no losses due to anaplasmosis among vaccinated cattle while 23 of the controls (45%) died of the disease.

Proposed Mechanism of Protection Induced by the Attenuated *A. marginale* Vaccine.

It has been shown that the attenuated vaccine induces both humoral and cell-mediated immune responses. Sera from convalescing animals are active in precipitation, agglutination, complement fixation and fluorescent antibody tests. Transfer of sera from immune cattle to susceptible animals, however, did not confer protection against anaplasmosis.

Cell-mediated immunity (CMI), as shown by *in vitro* tests developed 2 to 4 weeks following vaccination with the attenuated *A. marginale* (Figs. 5 and 7). These cattle were solidly protected when challenged with the virulent *A. marginale*. When susceptible animals were first inoculated with the virulent organism, a significantly elevated CMI response (see statistical analysis below) usually coincided with the development of clinical signs of the disease (Fig. 6). Once these animals were treated and recovered from the acute phase of the infection, a strong CMI response became evident and they were then clinically protected. This finding indicates a correlation between CMI and protection as well as the need for a gradual or balanced host-parasite interaction before protective immunity may be achieved.

Leukocyte migration inhibition (LMI) and lymphocyte transformation (LT) tests using peripheral blood leukocytes were employed for *in vitro* measurement of CMI. In view of the fact that the LT but not the LMI test rendered positive results following inoculation of inactivated antigens, and such animals on challenge exhibited a limited degree of protection, we concluded that the LMI test is a more accurate indicator of protective immunity. Animals inoculated with attenuated and virulent agents failed to develop cutaneous delayed hypersensitivity while less protected animals inoculated with inactivated-adjuvant fortified *Anaplasma* developed a delayed cutaneous hypersensitivity. Recent studies showed that such cutaneous reactions were due to erythrocytic antigenic determinants rather than to *Anaplasma* antigens.

While there is no indication of protection by antibody *per se*, opsonins of the IgG type were eluted from erythrocytes of infected animals and shown to sensitize autologous and homologous bovine erythrocytes to phagocytosis by an *in vitro* test. The period of maximal opsonic activity coincided with the time of anemic crisis and erythrophagocytosis in the bone marrow (53). Thus, the participating role of antibodies for *in vitro* protection is indicated. Under the circumstances, one could visualize that the protective immunity in anaplasmosis is an expression of participation of humoral and cell-mediated immunity, with the latter assuming the principal role. Upon

contact with antigens of the virulent organism, sensitized lymphocytes of vaccinated animals would induce activation of phagocytic macrophages and these in turn, aided by opsonizing effects of antibodies, rapidly remove infected erythrocytes from the circulation.

Statistical Analysis

Combined analysis of variance and T tests on LMIT data representing 0 to week 8 post injection (PI) for 4 cattle in each of the treatment groups, a) attenuated vaccine, b) premunized, c) inactivated vaccine, and d) susceptible controls generated the following information: The highly significant ($P = .01$) interaction of times with treatments makes it necessary to compare treatments at specific times rather than without regard to changes with time. The effect of the various treatments and associated changes with time were highly significant ($P = .01$).

The following comparisons are based on T tests using the error means square from the general analysis of variance and the appropriate numbers of observations per mean: (1) In cows immunized with the attenuated vaccine LMIT values increased slightly by the week (4) PI and exceeded baseline levels during weeks 6 through 8 PI to a highly significant extent ($P = .01$); (2) there was no significant change in LMIT values during the first 8 weeks PI in cattle which received the inactivated vaccine. The slight rise in inhibition levels for different cows occurring at various times established no general trend; (3) cattle infected with the virulent organism, i.e., premunized (treated) and controls (not treated) showed no significant ($P = .05$) increase in LMIT values until week 8 PI; (4) percent inhibition (LMIT) for cows having received the attenuated vaccine exceeded that of cows immunized with the inactivated vaccine by a highly significant ($P = 0.1$) degree in the 6th through 8th weeks PI although earlier differences are not significant ($P = .05$); (5) in the 7th and 8th weeks PI, premunized cows and those which received the attenuated *A. marginale* had similarly high levels of inhibition.

Economic Aspects and the Role of the Attenuated *A. marginale* Vaccine in the Prevention of Anaplasmosis.

Throughout tropical and semitropical regions of the world, anaplasmosis is considered one of the most important diseases of cattle. Although the mortality rate due to anaplasmosis is staggering, it is minor as compared to weight, milk, and calf losses. In addition, expense of treatment, special management and palliative convalescent care further adds to the economic burden caused by anaplasmosis.

Based on reports submitted by practicing veterinarians, a recent publication of the U.S. Department of Agriculture estimates that 50 to 100 thousand annual cattle losses can be attributed to anaplasmosis. These losses, combined with morbidity effects, are estimated at 100 million dollars a year (65).

South of the U.S. and throughout the Latin American continent, the economic impact of anaplasmosis is much greater. There are numerous enzootic areas in which losses due to the disease are experienced throughout the year. Cattle raised in these areas develop anaplasmosis during early calfhood and those transported from tick-free areas become ill shortly after introduction. Based upon our preliminary observation of the effect of anaplasmosis on cattle brought from northern México (Sonora) into southern endemic regions (Yucatan, Tabasco, or Veracruz), we estimated that translocation of 100,000 cattle will produce losses in excess of 20 million dollars.

All developing countries are striving toward exploring and developing new grazing land for expansion of their cattle industries. Most tropical jungle terrain is readily available and used to develop new pasture land. Modern technology, however, has not developed effective means for removing arthropod vectors from these newly-developed cattle raising areas. Under the circumstances, anaplasmosis constitutes a major threat to the private and government cattle investments in these areas.

Although effective chemotherapeutic compounds for treatment of anaplasmosis are now available, field experience has shown that immunoprophylaxis is the only feasible means of controlling the disease in enzootic areas. In these areas, application of the attenuated *A. marginale* vaccine would augment the development of the livestock industry.

The authors acknowledge the assistance of Dr. A. J. Lee, Department of Dairy Science, University of Illinois, in the statistical analysis of data.

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A REVIEW OF THE KNOWLEDGE OF THE VECTORS OF BOVINE ANAPLASMOSIS

*Kenneth C. Thompson**

A resurgence of studies on the biological relationships and transmission of *Anaplasma* evidences a new wave of popularity. It is now conceded that Smith and Kilborne, in their original classic demonstration of tick transmission of bovine piroplasmosis in 1893, observed *Anaplasma marginale* bodies as "peripheral coccus-like" bodies in erythrocytes of some animals. Ristic (1960) stated that "the exact means by which anaplasmosis is perpetuated in nature is little understood" a statement which probably still holds true today.

Experimental and epidemiological evidence incriminates horseflies (*Tabanus* spp.) as the most significant insect vector of anaplasmosis (Ristic, 1968; Roberts et al. 1968). Transmission by horseflies is effected only by mechanical means and usually must take place within a few minutes. Wilson and Meyer (1966) demonstrated transmission of bovine anaplasmosis from a diseased calf to an adult cow by immediate transfer feedings of the horsefly, *Tabanus fuscicostatus*. They further stated that some correlation between high horsefly population densities and the incidence of anaplasmosis existed in Louisiana. At least 10 species of *Tabanus* in the United States have experimentally been shown to transmit anaplasmosis (Ristic, 1968).

Other flies such as stableflies (*Stomoxys*), deerflies (*Chrysops*), black flies (*Simuliidae*), and hornflies (*Siphona*) are also potential vectors. Mechanical transmission has been demonstrated experimentally with mosquitoes of the genus *Psorophora* and the genus *Aedes*.

Roberts and Love (1973) reported that the eye gnat (genus *Hippelates*) can harbor the organism for at least three days. The opportunity for transfer through regurgitated or defecated material is greater than that of biting flies which can transmit mechanically only by interrupted feeding and then usually only within a very short time.

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Probably the most common method of mechanical transmission is by the cattleman using contaminated instruments (i.e. during dehorning and castrations) and needles (i.e. using the same needle to vaccinate all animals).

Howarth and McNeal (1973) stated that hematophagus insects are undoubtedly responsible for some transmission of anaplasmosis under rangeland conditions but that they should not be considered vectors of major importance. They assumed that ticks were most likely to be the only natural vectors capable of maintaining exposure to *Anaplasma* infection at the high levels demonstrated in their experiments in California, USA. Under the conditions of their controlled exposure trial, there was no evidence of flying insect transmission to, and infection of, platform housed cows by *A. marginale*. However, as they stated, no determination of insect activity on the platform was made.

Ristic (1968) reported that at least 20 species of ticks are capable of transmitting anaplasmosis under experimental conditions. He stated that the fact that transmission was effected by certain ticks under experimental conditions does not necessarily mean that the tick is a vector in nature. Ecological factors which include terrain, climate, vegetation, host predilection, tick habits, and their geographical distribution in their natural environment undoubtedly produce great variations in their roles as anaplasmosis vectors. Both argasid and ixodid ticks of several genera in the Old World and the New have been shown to be vectors.

Philip (1963) aptly pointed to the complexities and variety of acarine adapted agents, ie., viruses, rickettsiae, spirochetes, bacteria proper, and protozoa, plus noninfectious agents which give the Acarina the dubious honor of being the most versatile vectors and annoyers. This is not difficult to understand for ticks and mites have been living at the expense of animal hosts for a much longer period in modern geologic history than have the more recently evolved biting flies. Many ticks have become specifically adapted to certain hosts and their habits, and most ticks are parasitic in all stages after the egg.

Ticks may function as more effective reservoirs in many pathogenic ecosystems than more highly organized parasitic insects, and even many affected vertebrates. Several factors which favor ticks as reservoir host include: "longevity; resistance to starvation and overwintering or inter-epizootic survival in appropriate environments; frequent attack on more than one vertebrate host in a given generation; adaptations that result in transmission by several stages and passages of agents through the eggs to progeny; plus cyclic propagation in the vectors".

Philip (1963) further stated that there is a tendency to attribute advantages as vectors to parasites with wings, particularly mosquitoes, which appear to be more mobile in seeking hosts. But the advantage recedes when it is recalled that (1) that ticks, especially many of the argasids, are much more durable, and (2) that many hosts of Acarina not only travel farther than fragile biting flies but may also transport all active acarine stages. Ticks may even transgress continents on migrating birds and bats, or on exported animals.

Passage of pathogenic organisms from one tick generation to the next through the eggs probably represents the highest degree of adaptation to residence in the vector, particularly among the viruses, rickettsiae, and spirochetes. The bacteria causing tularemia are irregular in this regard. Noteworthy is the transovarian passage of the protozoan, **Babesia**.

An important advance in the modern study of acarology is the growing realization that more than morphological criteria are needed to delineate many pathophorous* species which display puzzling geographic differences in biologic activities.

An example is the ubiquitous brown dog tick, **Rhipicephalus sanguineus**, whose habits of attaching and transmitting pathogens to man in certain parts of the Old World, Kashmir, and northern Mexico are lacking in other areas of abundance, e.g., southern United States (American spotted fever). On morphological grounds Feldman-Muhsam (1952) in Palestine separated a second species, **R. secundus**, which may in part explain differences in reported habits. When Hoogstraal (1956) pointed out population differences under varying environmental conditions and host predilection (in part based on studies of Walton), he called attention to similar discrepancies in our supposedly well-rounded knowledge of the African relapsing fever vector and the human parasite, **Ornithodoros moubata**. Theiler (1947) contrasts the ungulate host propensities and vectorship of the **Hyalomma** species in USSR with their failure to infest even domestic stock in South Africa. There are many taxonomic problems still to be solved that transcend strict morphological criteria and impede confident delineation of pertinent vector relationships.

Interference phenomena between animal pathogens are now well known but are little assessed in ticks concomitantly infected with more than one agent; yet this could be an important factor in vectorship in certain biocenoses. No effect on the transmissibility of simultaneous

* Greek path- a prefix denoting suffering or illness, and phero, to bear or carry; in contradistinction to "pathogenic", to cause disease (applied to the etiologic agents). "Viruliferous" is an adjective employed by plant virologists to describe infected vectors.

infections in the same ticks with *Anaplasma* and *Theileria* was noted in the reports of Bitukov (1953) and D'yakonov (1959), but the problem merits further investigation of other agents.

Neitz (1956) in his consolidation of the knowledge of the transmission of tick-borne diseases stated that transovarial transmission of anaplasmosis occurred in the *Boophilus* spp., in *Dermacentor andersoni*, *Haemaphysalis cinnabarina punctata*, *Hyalomma excavatum*, *Ixodes ricinus* and *Rhipicephalus simus*. In the remaining ticks, *D. albipictus*, *D. variabilis*, *Rh. bursa*, *Rh. sanguineus* and *Argas persicus*, only stage to stage transmission within the same generation has been recorded.

However, as is known, the eradication of *B. annulatus* and *B. microplus* over a large area in the United States of America was followed by the eradication of babesiosis but not by that of anaplasmosis (Ristic, 1968). *Argas persicus*, *D. variabilis* and *Rh. sanguineus* are rarely found on cattle. *D. andersoni* is more or less limited to the Rocky Mountain States, and *D. occidentalis* to California. The geographical distribution of *D. albipictus* extends beyond the enzootic anaplasmosis area. In the case of *I. scapularis* the seasonal occurrence of the adults (the only stage that feeds on cattle) cannot be correlated with the seasonal occurrence of the disease. Hence, it appears mechanical transmission is responsible for anaplasmosis in these areas.

Howell (1957) and Reshetnyak et al. (1956) also reviewed arthropod transmission of anaplasmosis. Reshetnyak et al. incriminated three additional ixodids in the USSR. Bitukov (1953) first reported transmission in Kazakh by adult *H. sulcata* and by larvae and nymphs of *O. lahorensis*, but his protocols are unclear as to whether transovarial passage was involved. D'yakonov (1959) reported apparent natural infection of *R. turanicus* in the Stavropol region, USSR, adults of which infected a ram on transfer. This could not be asserted as more than a mechanical transmission.

The presence of ticks other than *B. microplus* and of several associated tick-borne diseases is an added complication in tropical areas outside Australia, however, Seddon and Albiston (1966) stated that in Australia *Anaplasma marginale* infection of cattle has been found only in cattle that have been infested with the cattle tick *Boophilus microplus*. Rogers (1971) suggested that although anaplasmosis is of secondary importance to babesiosis in Australia, the number of outbreaks of anaplasmosis in Northern Queensland is significant.

It has been stated by Springell (1974) that Australia is fortunate in having only one tick species of major economic importance. If this

is the case it would be of interest to see if anaplasmosis occurs in areas of Australia which are outside of the **B. microplus** belt.

Kuttler et al. (1971) using unfed larvae of **Boophilus annulatus** reported successful transovarial transmission of anaplasmosis to two splenectomized calves, but recent work using a different species i.e. **B. microplus**, shows the contrary.

Leatch (1973) reported that while **A. marginale** was readily transmitted by transfer of stages of **B. microplus** the following generations of the same ticks were incapable of transmitting infection. A number of factors such as conditions of incubation of females during egg-laying and of larvae may be involved. In addition, some strains of **A. marginale** or of **B. microplus** may modify the incidence of this type of transmission.

In experiments by Connell and Hall (1972) interrupted feeding and removal to a susceptible host and transfer of the next instar were the successful methods of transmitting **A. marginale**, whereas six attempts at transovarial transmission by **B. microplus** were unsuccessful.

Uilenberg (1973) summarized his work of 1968 and 1970 by stating that all attempts to transmit anaplasmosis transovarially by **B. microplus** had failed even though all epizootiological evidence pointed to its role as the vector. An infected animal was then infested with larvae of **B. microplus** and penned 4 days later with a susceptible splenectomized steer, free of ticks. This steer acquired ticks from the first animal, and became infected with **A. marginale**.

Summary Questions

1. How important is **Boophilus microplus** in maintaining anaplasmosis in nature when: (1) its ability to transmit anaplasmosis transovarially is questionable; (2) it is a one host tick (although as shown above, some transfers to other hosts do occur)?

2. How important is a biological vector (the organism undergoing some change, development or multiplication) to perpetuate anaplasmosis in nature?

3. Does elevation itself effect the transmissibility of the **A. marginale** organism or is it the absence of the vectors which is the criterion?

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Other contributions

EPIZOOTIOLOGY OF ANAPLASMOSIS AND BABESIOSIS IN URUGUAY

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The genera **Babesia** and **Anaplasma** have existed in Uruguay since the turn of the century. The first law on sanitary control established on the 13th April 1910 already included "bovine tristeza" but the exact date of the first diagnosis is not known. During the twenties (1924), Rubino successfully attempted to infect sheep with **Babesia**.

The existence has been reported in Uruguay of different genera of hematophagous insects and ticks important to the epizootiology of the hemoparasites with which we are here concerned (Castro and Trenchi, 1955). Outbreaks of anaplasmosis have been reported that were associated with **Stomoxys calcitrans**, different species of tabanids, hypodermic needles and several surgical procedures (Carballo Pou, 1948). However, it has been generally accepted that **Boophilus microplus** is the principal vector of the genera **Babesia** and **Anaplasma**. **Ixodes ricinus** also exists but from the time of the research of Rubino and Varela Calzada (1943) to the present date there has been no outbreaks of babesiosis associated with this tick. The time of the first appearance of **Boophilus** in Uruguay is not known, but, as already mentioned, the sanitary measures existing at the start of the century recognized its presence. For several decades, therefore, workers at the Centro de Investigaciones Veterinarias have attached fundamental epizootiological importance to **Boophilus microplus**.

This was the background to the first law for the eradication of ticks in Uruguay (1940). The country was divided into two sectors North and South. The division was made because of the limited number of outbreaks of tick infestations in the South, contrasting with the situation in the North where **Boophilus** was enzootic. Incidentally, outbreaks of anaplasmosis and babesiosis were observed almost exclusively in the northern sector.

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Recently, *Boophilus microplus* has become enzootic in areas that were previously considered "clean". Furthermore, there have been repeated outbreaks of babesiosis in the southern sector.

What, then, are the chances for the spread of hemoparasites in the country? We can here introduce the concept of break-down in epizootiological balance which can happen in areas marginal for ticks or in which tick populations have been artificially reduced (Albiston, 1966). Relative to Australian information (McCulloch and Lewis, 1968) the climate of Uruguay would make it a marginal area. After his visit to Brazil in 1973, Lewis reported that, as Uruguay is below the 30° parallel South, given a similar climate to Australia, the whole country would be marginal. At the Wollongbar Experimental Station, 29° South, only four *Boophilus microplus* tick generations have been reported in one year. On checking the ranges of mean temperatures in Uruguay, it can be seen that in mid-winter no more than two degrees difference exist between Rivera and Montevideo. If this data is compared to that published by McCulloch and Lewis (1968) for Wollongbar a great similarity is observed although temperatures are generally lower in Uruguay between March and October. The authors have stressed the importance of the humidity factor since it is necessary to have a 70% minimum relative humidity for the hatching of *Boophilus microplus* eggs (Hitchcock, 1955). This point is interesting in that the average rainfall for the last 50 years increases from 1000 mm. in Montevideo to 1500 mm. in Rivera and is uniformly spread throughout the the year. In Wollongbar the annual average is 1600 mm. (Table 1). These data are interesting in

Table 1. Comparative data for areas of Australia (Wollongbar and Pretty Gully) with areas in Uruguay.

Place	Latitude	Altitude	Rainfaull
		M	mm
Wollongbar	29	130	1.550
Pretty Gully	29	600	1.350
Rivera	30°54	280	1.508
Treinta y tres	33°11	31	1.150
Montevideo	34°52	22	1.018

comparison with locations that are considered marginal according to McCulloch and Lewis (1968). Pretty Cully lies at 600 m. above sea level whereas in Uruguay there is no elevation greater than 500 m. It was in Pretty Cully that the authors reported faults in the oviposition of many of the fallen ticks and lack of hatching of eggs laid from early March to late August. In Wollongbar, hatching is possible all year round, excepting May and June. In spite of little available information, it is accepted that the bionomics of the Uruguayan tick is not regulated by any fixed pattern. As was previously stated, the knowledge of the bionomics of **Boophilus** acquires fundamental importance in marginal areas. Because of this fact, studies were started at the Centro de Investigaciones Veterinarias on tick prevalence and bionomics, and also including acaricide resistance.

Returning to the Albiston concept (1966) it may be presumed that the epizootiological status of the **Babesia** and/or **Anaplasma** species is also unstable. In our country we cannot yet adopt the Mahoney and Ross philosophy (1972) to differentiate areas of greater or lesser risk. Studies on the distribution and bionomics of the tick are therefore being carried out at the same time as serological surveys for **Babesia** and **Anaplasma**.

It is recognized that the prevalence of these hemoparasites is not an exclusive function of the tick distribution. Other factors exist which could be fundamental to their epizootiology. The cattle population, for example, is exclusively **Bos taurus**.

The local market and export trade, principally to Brazil and Paraguay, make use of a vaccine derived from infected blood. Uruguay has an official laboratory which supplies blood infected with **Anaplasma centrale**, **Babesia bigemina** and **B. argentina**. The existing legislation, however, is not sufficiently clear to stop private, non-technical personnel from handling sources of supply. These practices cause frequent failures in the protection of vaccinated animals leading to the general practice of challenging such animals with strains from the place of destination. It is common, therefore to inoculate animals, in Uruguay, with blood from Brazil, Paraguay or Argentina. These situations are often unknown to officials and only the producers involved know what is happening.

In 1968 the foot and mouth disease campaign was initiated. It required that all producers be obliged to vaccinate their cattle three times a year, at periods officially established. These inoculations, not always carried out by technicians, could result in the propagation of anaplasmosis. In 1973 the Diagnostic Laboratory of the Centro de

Investigaciones Veterinarias found that two *Anaplasma* outbreaks in the northern sector were due to faulty inoculation.

In addition, in spite of *Babesia bigemina*, *Babesia argentina* and *Anaplasma marginale* being diagnosed in the country, prevalence studies were never made. For many years different work groups accepted that *Babesia argentina* was practically restricted to the Paysandú area. During Dr. Callow's visit in July 1974, infection was confirmed in the same area but in a place previously thought non-infested. Later, diagnosis was made of acute cases associated with *Babesia argentina*, in the states of Cerro Largo and Treinta y Tres, during August and September.

Starting in 1974, a technical assistance project of the UNDP has made the development of the Parasitology Department possible in the Centro de Investigaciones Veterinarias. It is hoped that research in this field can be intensified, within the philosophy of parasitism as a regulating factor in livestock production.

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COMMENTS ON THE DIAGNOSIS OF BABESIOSIS

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I must congratulate Dr. Mahoney for his excellent keynote paper. For many years now, work in Britain on babesiosis has lagged behind that in other countries, principally Australia, because it is not a big problem for us. Barnett (1974) estimated that redwater was probably costing Britain about £400,000 a year. This is not a negligible amount but looks small when set against figures like £50,000,000 for liver fluke.

In this workshop we are discussing control and eradication and two methods of diagnosis which may be useful - smears and serological tests. The use of smears is well known to us all but Dr. Mahoney has given us some useful rules for their application under field conditions. Smears are certainly the most important technique for the recognition of infection although in Britain and other European countries diagnosis is invariably based on clinical observation. Redwater used to be the disease that teachers in veterinary school would say could be diagnosed over the telephone - so loud is the thumping of the animal's heart. A second common diagnostic method, of course, is response to treatment. If the animal responds to the administration of a babesicide, it was redwater. I agree that thick films should be used more often but there is a general reluctance to move away from the familiar thin film technique. In England I have had trouble using the thick film method for *Babesia divergens*, but no difficulty with the much larger *Babesia major*.

I, myself, am not a serologist so that I was delighted to see Dr. Tododovic's recent review entitled "Serological Diagnosis of Babesiosis" in a recent copy of *Tropical Animal Health and Production*. This is a most valuable and up-to-date account of the subject. However, one thing I have noticed is that people become astonishingly attached to a particular technique and some can hardly bear to talk about any other method. This leads me to suppose that there is no perfect test available.

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Let me now ask a naive question. Do we need a serological test to assist us in control or eradication? I suppose that the two best known examples of the eradication of piroplasm diseases of cattle are the successful campaigns against Texas Fever in the United States and the defeat of East Coast Fever in South Africa. Both were accomplished without any serological aids. Of course, serological tests have many applications but their use in the fields of control and eradication needs to be argued.

There is another danger that is often ignored which I can illustrate with two examples from outside the babesiosis field. The first concerns a survey recently carried out in Kenya by an FAO project, KEN 22. Mainly using the Indirect Hemagglutination and Indirect Fluorescent Antibody tests, the team conducted a countrywide survey on the incidence of East Coast Fever, and attempted to correlate the serological results with the answers that were given by stockowners. At this moment the results of the survey have not been published but I do not think I am breaking any confidences when I tell you that there were significant numbers of reacting cattle in districts that were well known to be free from the disease - and stockowners claimed they had no tick problems whatsoever.

The second example concerns an extensive abattoir liver fluke survey recently carried out in Britain by a commercial firm. It was formerly thought that fluke was, like *Babesia*, mainly restricted to the western parts of Britain. This new survey, which has been heavily publicized, showed that infected cattle were present throughout the country. Infected cattle were found on almost every farm.

Both these surveys achieved much, but they suffered because they took no account of cattle movements. This is a simple fact which is often ignored. Beef cattle populations are extremely mobile. In Britain such cattle stream from West to East bringing their liver flukes and presumably, their babesial antibodies with them.

The point I am getting at is that we should pay more attention to vectors which, when compared with cattle, are relatively static. The tick stages of *Babesia* are large and easy to recognize. At Compton we have found that egg squashes reveal stages of *B. major* quite readily. Perhaps we should collect adult ticks from the vegetation, allow them to feed on experimental animals and then examine their eggs. In this way we would learn about the infection rates in the local ticks. Perhaps the Feulgen method developed at the Centre for Tropical Veterinary Medicine, Edinburgh, could play a part in this.

In conclusion, then, let us consider the possibility that we may be paying a little too much attention to antibodies in cattle and not enough to the parasite in the tick.

EVALUATION OF PREMUNITION FOR THE CONTROL OF ANAPLASMOSIS AND BABESIOSIS ON COMMERCIAL RANCHES IN THE CAUCA VALLEY (COLOMBIA)

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Introduction

For many years, in Colombia in particular, bovine anaplasmosis and babesiosis have been a constant problem and a cause of economic loss. However, their effective control is still a large question in this country where, for many reasons, an eradication program of vectors is virtually impossible. Recently, several trials have been carried out under experimental and field conditions, to test different methods of control of these diseases. The premunition and chemoprophylaxis program have shown good results although there are still problems to be solved, especially in the applicability to field conditions.

The concept of "coinfectious immunity" or "premunition" has been known for several years and has been and is being practiced, by various means, in the countries of the tropics where anaplasmosis and babesiosis exist.

Premunition has been practiced in Colombia by several breeders, as a means of protecting their calves when they are first put out in the field. The simplest method consists in taking blood from an adult, preferably the calf's mother, and injecting it into the calves before letting them out to pasture. There are other methods used on other ranches as a routine practice. Although it is true that these methods have reduced the problem on some ranches, it still persists in some areas, especially on dairy farms as problem number one.

In South America, the geography and ecology of the diseases are similar in many countries that are situated in the tropics where there are areas of different altitudes above sea level. The western region of Colombia, the most developed, is crossed by three ranges of the Andes, where altitudes vary from 0 to more than 4,000 meters. Great

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valleys run between these ranges, reaching heights of 800 to 1,500 m, and whereas anaplasmosis and babesiosis are endemic in them as in the North coast and the Eastern plains, here they present a larger problem because cattle breeding is more advanced with European stock and different management procedures. There are also areas of over 2,500 metres, such as the Sabana de Bogotá and the highlands of Nariño, where the diseases are practically non-existent. This situation restricts the movement and rearing of herds in certain areas, and causes many anaplasmosis and babesiosis outbreaks in the intermediary areas. The presence of these diseases is also a limiting factor in the importation of stock and the establishment and development of other breeds.

In light of these considerations and of the results obtained in previous tests, it was thought that a premunition program should be started in Colombia, on commercial ranches, to test its applicability under field conditions. However, in this country, anaplasmosis and babesiosis appear as concurrent infections, making impossible a unilateral control of either one. A premunition programme in Colombia should, therefore, be directed to control the *Anaplasma marginale*, *Babesia bigemina* and *Babesia argentina* parasites together, since, according to epidemiological studies carried out up to this moment, they are of great pathological significance.

This is why it was decided to initiate a cooperative project between the special Texas (TAMU) project at the Centro Internacional de Agricultura Tropical (CIAT), and the Instituto Colombiano Agropecuario (ICA), to evaluate the results of premunition in the field, carried out on ranches in the geographic area of the valley of the Cauca River. The principal objectives of this programme are as follows:

1. Develop and evaluate various premunition techniques under experimental and field conditions;
2. In large scale trials, determine whether the methods that have shown best results on a small scale, are effective in reducing, to a minimum, the losses caused by anaplasmosis and babesiosis in commercial ranches;
3. Compare the effectiveness of premunition in pre-immunized animals with non-pre-immunized animals, in terms of weight gain, age of maturity, efficiency in reproduction and production;
4. Determine by cost analysis whether this program is economically beneficial under commercial conditions.

Up to the present moment 12 ranches have been visited in the states of Valle and Cauca and, in cooperation with animal health

veterinarians from ICA, samples have been taken, of different age groups, with the purpose of determining prevalence according to age, management conditions, types of ticks and problems caused by babesiosis and anaplasmosis. The ranches were chosen according to their geographical situation, number of cattle and their hemoparasitic problems. It should be noted that premunition program are already being carried out on some of these ranches and, in cooperation with the ranchers and veterinarians, a comparison will be made between the different premunition methods employed.

Management systems and problems are different on each ranch and it is therefore necessary to establish an area diagnosis before initiating any program. The most common management practice used on dairy farms is that of stabling the calves until they are 4 to 5 months old and then letting them out to pasture or semi-pasture, up to the age of 10 to 12 months. Under these conditions, problems occur when the calves go out to graze. They start suffering from infections; some die and others are considerably retarded. This factor of retarded development is important because the animal has difficulty in recuperating. The stage at which to practice premunition is, therefore, when the animals are stabled, before being let out to pasture.

Another situation exists on ranches in the mountain region. The case here is dramatic since the ranchers cannot move their herds to the valley areas and, when they are forced to do so, they lose more than 50%. Furthermore, in the aspect of economics, the price of a cow from the mountain region is much lower than one from the valley but, due to the limiting factors caused by these diseases, ranchers cannot profit by marketing their stock. Under these conditions a premunition program can be practiced at any age, before moving cattle to the endemic zone.

Laboratory Work

The premunition program to be carried out is based on the principle of the use of stabilates, prepared with *Anaplasma marginale*, *Babesia bigemina* and *Babesia argentina* organisms, isolated and purified in Colombia. Stabilate is the term used for any biological substance that is preserved in certain conditions under which it does not lose its viability. In this case the organisms are preserved at -65°C in dry ice. These organisms are injected into splenectomized calves, selected for their excellent health and good hematocrit value. When a good parasitemia is obtained, with a hematocrit value of not less than 20%, the animals are bled by canulation. The blood that is drawn is centrifuged to separate the plasma, then it is washed twice in a sterile solution of buffered phosphate (pH 7.2) and the concentrated

blood is diluted, in equal quantities, with 4M-DMSO, as a cryoprotective. It is then placed in phials and immediately frozen in liquid nitrogen for two hours. The blood is kept in a special container, with dry ice, at -65°C , until required. Parasite counts are made before dilution with DMSO. Titration of the stabilates was carried out in groups of three animals, using splenectomised control and two intact animals. For *A. marginale* we used dilutions up to 10^{-5} , finding 10^{-3} to be the most appropriate.

Of this dilution, 2 cc. are utilized intravenously, containing 2.6×10^6 organisms of *A. marginale*. Under these conditions an average parasitemia of 10% was obtained, a reduction in hematocrit value in average of 17% and no treatment was given. The animals recuperated without treatment.

As a guide in calculating the number of doses that can be obtained for use in premunition, if a calf of about one year of age is used, up to 8 litres of blood can be drawn, giving about 2,000 cc. of red corpuscles which, diluted with 2,000 cc. of DMSO will give 4 litres of stabilate. From this, 1 cc. is diluted with 999 cc. of PBS, to obtain one litre of vaccine. Therefore, from 1 cc. of stabilate, 500 doses are obtained, ie. 2,000,000 vaccine doses, from one young calf. Economically, one dose would cost a ridiculously low sum.

In the case of *B. bigemina* and *B. argentina* it was found that a dilution of 10^{-1} produced a good grade of protection, utilizing 4 cc. of this stabilate containing 10^8 *B. bigemina* organisms and 10^8 *B. argentina* organisms, per inoculant. When blood diluted with *B. argentina*, containing 10^8 organisms was used, it became necessary to apply treatment to counteract the infection although, in the case of *B. bigemina* with diluted blood, no treatment was required.

Field Work

For their application in the field, stabilates are removed and kept in a small thermos flask containing dry ice, until arriving at the ranch where premunition is to be practiced.

A simple field kit has been prepared for persons to carry the stabilates and dilutants. The kit consists of 2 thermos flasks, one with dry ice for the stabilates and one with common ice for the samples to be drawn from the animals to be premunized. It also includes tools for handling the animals.

On the ranch, the stabilates are unfrozen in ordinary hot water, a process that requires no more than 5 minutes, and then the dilutants corresponding to each organism are prepared, in this case, a dilutant of 10^{-3} for *A. marginale* and 10^{-1} for *B. bigemina* and *B. argentina*.

Once the dilutions have been made they are mixed in correct quantities which contain 10^7 *Anaplasma* organisms and 10^8 of each of the *Babesia* organisms. This is possible by mixing approximate amounts of each organism which produce a final inoculant containing 2 cc. of *A. marginale* and 4 cc. of each of the *Babesia* organisms, for a total of 10 cc. of vaccine for intravenous application, per animals.

This has been a summary of the premunition program that will now be evaluated, under field conditions, on commercial ranches in the Cauca valley.

THE TEXAS A&M MONTERIA EXPERIMENTS

NUMBERED I, II, III AND IV

(Abstract)

*T. J. Galvin**, *L. G. Adams**, *Guillermo Mateus***

The experiments were carried out at the Turipaná Experimental Stations of the Colombian Institute for Agriculture (ICA) near Montería on the north coast of Colombia.

In the first experiment, calves from a "clean" area for *Anaplasma marginale*, *Babesia argentina* and *B. bigemina* were introduced into the severe endemic challenge found at Turipaná. The groups compared were: premunition plus anthelmintics; chemoprophylaxis (imidocarb dipropionate) plus anthelmintics; anthelmintics alone, and control animals. Both premunition and chemoprophylaxis gave important degrees of protection. Following the experience gained of the logistics of this experiment, three further field experiments were designed and started, the total sequence being designated Montería I, II, III, and IV.

Montería II compared chemoprophylaxis (imidocarb dipropionate), chemotherapy (imidocarb dipropionate) and premunition for the control of hemoparasites in susceptible Normando calves introduced from a "clean" area (Sabana de Bogotá). Montería III complemented Montería II and compared only premunition and chemoprophylaxis in a group of susceptible Holstein calves. Montería IV was designed to determine whether any advantage could be gained from immunizing calves born in the highly endemic Turipaná area. The definitive results are being assessed, but in general, the hemoparasite control measures resulted in decreased mortality and increased weight gains in introduced calves, but were of no economic benefit in calves native to a highly endemic area. The conditions included a strict regime of control of gastrointestinal and pulmonary parasites and moderate control of external parasites.

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WORKSHOP RECOMMENDATIONS

WORKSHOP RECOMMENDATIONS

Chairman : Dr. Eddo Caletti

1. Epizootiological studies of babesiosis and its economic impact should be carried out in every Latin American country using the card, passive hemagglutination, and fluorescent antibody tests.
2. Epizootiological studies of anaplasmosis and its economic impact should be carried out in every Latin American country using the card, capillary agglutination, or complement fixation tests.
3. CIAT should become the center for documentation, terminology, and training, for professionals in Latin America.
4. There should be an intensification of studies in the methods of control of babesiosis in each Latin American country, using local strains for each species of **Babesia**.
5. There should be an intensification of study in the control of anaplasmosis in each Latin American country, using either mild or attenuated strains.
6. There should be studies in the distribution and significance of vectors of babesiosis and anaplasmosis, particularly in their capacity as reservoirs of infection, in each Latin American country.
7. Information should be gathered on the development of acaricide resistant ticks in Latin America. The information should be channeled through CIAT for distribution.
8. There should be further seminars or workshops on subjects raised during the present sessions.
9. There should be a standardization of antigens and antibodies used in research on anaplasmosis and babesiosis.
10. F.A.O. should be asked to assume responsibility for the formation of a reference bank for the following antigens and antisera:
 - a. **Babesia** species
 - B. bigemina**
 - B. argentina**
 - B. major**
 - B. divergens**

- | | |
|-----------------------------|--|
| b. <i>Anaplasma</i> species | <i>A. marginale</i>
<i>A. centrale</i>
<i>Paranaplasma caudatum</i> |
| c. <i>Theileria</i> species | <i>T. parva</i>
<i>T. annulata</i>
<i>T. mutans</i>
<i>T. lawrencei</i> |

The reference bank should be available to all interested scientists. The maintenance and distribution of items should be through experts designated by F.A.O. in each case.

This proposal should be sent by CIAT to Dr. H. A. Jasirowski, Director of the Division of Animal Health and Production with a copy to Dr. R. B. Griffiths, Head of the Health service, both of F.A.O., Rome.

Addendum

At the end of the workshop the recommendations were sent to all delegates asking for any further comment.

Most delegates who replied accepted them without reservation but a few reminded the organizers of other suggestions which did not meet the approval of the meeting and therefore were not included in the final list.

For completeness, the additional suggestions and commentary are summarized as follows:

1. The Animal Health Group in CIAT should have a strong research input particularly in relation to innovations in vaccination.

2. Greater emphasis should be put on the collection of field data such as: the frequency of clinical outbreaks of babesiosis and anaplasmosis; geographical distribution; species of organism; the number and type of origin of cattle involved, and the losses sustained. That is, existing problems must be clearly defined.

3. A comparison should be made between the pathogenicity of the two species of *Babesia* present and also of the different field strains within each species.

4. The definition of the role of *Boophilus microplus* in the transmission of anaplasmosis deserves high research priority.

5. A recommendation is required emphasizing the importance of basic research on arthropod tissue culture particularly in relation to production of future vaccines and stablulates.

6. The recommendation on acaricide resistance should refer to desirability of collaboration with FAO in the establishment of a global acaricide resistance monitoring program.

Se terminó de imprimir este libro en
el mes de julio de 1977 en los Talle-
res Gráficos de Canal Ramírez - Antares,
Imprenta - Litografía - Fotomecánica.
Cra. 4ª N° 25B-50 - Bogotá - Colombia.

