

QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES  
DIVISION OF ANIMAL INDUSTRY BULLETIN No. 153

TRANSMISSION OF *ANAPLASMA MARGINALE* BY  
THE CATTLE TICK *BOOPHILUS MICROPLUS*

By MAXINE L. CONNELL, B.Sc.

SUMMARY

Experimental procedures were devised to determine the mode of transmission of *Anaplasma marginale* by the cattle tick *Boophilus microplus*.

Transmission of anaplasmosis was achieved by trans-stadial (that is, larvae-to-nymph; nymph-to-adult female; nymph-to-adult male) and intrastadial methods (that is, transfer of the feeding stage at mid-instar from an infected host to a susceptible, splenectomized recipient). Incubation periods with the ticks and *A. marginale* strains employed ranged from 26 to 47 days.

However, the transovarial mode of transmission proved negative in all trials, even though field and laboratory strains of ticks and *Anaplasma* were used. In these experiments, susceptible, splenectomized, recipient steers were infested with larvae, the progeny of adult ticks engorged on animals with *A. marginale* parasitaemias. No evidence of infection could be detected in the recipients.

I. INTRODUCTION

The role of blood-sucking Arthropods in the transmission of anaplasmosis was evaluated by Ristic (1968). He listed the work of Philip (1963) and others in which at least 20 species of ticks, as well as species of *Tabanus*, *Stomoxys*, *Chrysops*, *Siphona* and mosquitoes of the genus *Psorophora* were incriminated as vectors.

In Australia, *Anaplasma marginale* is considered to be transmitted by the cattle tick *Boophilus microplus* as the endemic *Anaplasma* area corresponds to the area of cattle tick infestation. Thousands of cattle infected with *Anaplasma* have been moved to tick-free areas without any evidence of transmission from infected to healthy cattle (Seddon 1966). Mackerras, Mackerras and Mulhearn (1942) were unsuccessful in attempts to transmit *A. marginale* with *Tabanus circumdatus* Walk. and *Stomoxys calcitrans* Linn.

Transovarial transmission of anaplasmosis by *B. microplus* has been described on a limited number of occasions by Theiler (1912); Quevedo (1916, 1929); Rosenbusch and Gonzalez (1927) and Brumpt (1931). However, other

workers (Lignières 1919; Brumpt 1920; Gomes de Faria 1928; Regendanz 1933) were unsuccessful with this type of transmission. Neitz (1956) quotes Legg (1933) and Mackerras, Mackerras and Mulhearn (1942) as indicating that transovarial transmission of *A. marginale* by the cattle tick may occur in Australia, but neither reported any experimental evidence. Uilenberg (1968) was unable, in 25 attempts, to demonstrate this method of transmission, as were Connell and Hall (1972) and Leatch (1973).

Regendanz (1933) queried whether *B. microplus* is a one-host tick in the field and suggested, from his observations, that the parasitic stages constantly change their site of attachment and therefore the one host *Boophilus* could transmit anaplasmosis without having to pass infection by hereditary means onto the subsequent tick generations. In Madagascar, Uilenberg (1970) was successful in transmitting infection from an animal infected with *Anaplasma* and infested with *B. microplus* to an uninfected animal kept in the same pen. He suggested that transfer of infected stages of *B. microplus* was responsible for the transmission of the disease. Connell and Hall (1972) reported 'in contact' transmission of *Anaplasma* and described unsuccessful attempts at transovarial transmission. Some successful trans-stadial and intrastadial (that is, interrupted feeding at mid-instar) transmissions were also reported.

This paper gives further details of these earlier results and reports subsequent experiments on the transmission of anaplasmosis.

## II. MATERIALS AND METHODS

**EXPERIMENTAL ANIMALS.** Animals used in these experiments were calves and steers of mixed breeds purchased from an area free from ticks and *Anaplasma*. Cattle were sprayed with Dursban 0.05% and then kept in tick-free paddocks to ensure freedom from *B. microplus* infestation at the commencement of experiments. Approximately one month prior to use, animals were splenectomized and serum samples taken at that time were tested to detect any *Anaplasma* carriers by (1) the capillary agglutination (CA) test (Rogers 1971), (2) the complement fixation (CF) test (Price, Poelma and Faber 1952) and (3) in later experiments by the Fluorescent Antibody Test—Fluorescent Antibody conjugates were prepared for this test from serum samples of animals recovered from patent parasitaemia. Globulins were separated from immune sera by precipitation with ammonium sulphate. The methods described by The and Feltkamp (1970) were used for the conjugation of globulins to Fluorescein Isothiocyanate (Baltimore Biological Laboratory, Inc.) and for the purification of the conjugate. Thin blood films were fixed in anhydrous acetone for 30 minutes, and then stained with conjugates as described by Marshall, Eveland and Smith (1958).

In experiments started before these tests were developed, thin blood films were taken during the holding period and examined for *A. marginale* bodies.

**STRAINS OF *A. MARGINALE*.** Two laboratory strains of *A. marginale* designated the A.R.I. strain and the Wacol strain had been isolated at this Institute and maintained here by animal passage and storage in liquid nitrogen. This material was referred to as the *A. marginale* stabilate. A field strain was also obtained from a naturally occurring infection.

**STRAINS OF *B. MICROPLUS*.** The laboratory strain of cattle tick, maintained at this Institute for 15 years, was *Anaplasma* free while the field strain was isolated from an area where anaplasmosis was endemic.

DETECTION OF *A. MARGINALE*. Infection was said to be present when *A. marginale* organisms were seen in at least 0.5% of red blood cells in a Giemsa-stained blood film.

### III. EXPERIMENTAL PROCEDURE

Calves were housed in tick-proof concrete pens, surrounded by a moat containing copper sulphate solution. Buffer animals were housed in pens adjacent to those of the experimental animals.

(a) TRANSMISSION BY TRANSOVARIAL TRANSFER. On day 1, 7, 11, 15 and 22 a donor calf was infested with 0.5 g of 7-day-old 'clean' *B. microplus* larvae and on day 7 was inoculated intravenously with *A. marginale* stabilate. About 2 days after each infestation, engorged female ticks, after having dropped from the host, were hosed from the floor of the pens into wire collecting baskets from which they were removed individually and then maintained in the constant temperature and humidity room (30°C, 90% R.H.) for egg laying and subsequent hatching of the eggs.

Recipient calves were infested with 1 g of 7-day-old larvae, the progeny of the females feeding on the host at a time when the parasitaemia was at a maximum. Thin blood films were examined three times a week to detect any parasitaemia and serum samples were taken weekly to be tested by the CF test. After female ticks had engorged and dropped, the host was sprayed with Dursban 0.05%. Recipient animals not developing a parasitaemia after infestation were challenged 90 days later with 5 ml of *A. marginale* stabilate.

(b) TRANSMISSION BY INTRASTADIAL TRANSFER. Similar penning arrangements to those previously described were followed in this trial.

Inoculation procedure was the same.

However, the method of serial infestation was superseded by a 'once-only' infestation procedure. That is, on the appearance of initial *Anaplasma* bodies in a thin blood smear stained by Fluorescent Antibody conjugates, cattle were infested with 0.5 g of clean larvae. In most cases, patent parasitaemia developed 3 days later. This infestation procedure required adaptation to suit the experiment involving the intrastadial transfer of larvae, because larval feeding time on the donor host was only 2.5 days. Infestation was therefore delayed until the bovine erythrocytes were showing a 2% parasitaemia in a Giemsa-stained blood film.

Larvae were confined to one site on the host by infesting under an organandie fabric patch, which was glued by a contact cement along its edges to a shaven area on the side of the animal. Ticks were removed at mid-instar and, after microscopic checking for the stage of development, were transferred to a patch on a recipient animal. A scalpel blade was used to scrape larvae from the donor host, while nymphs and adults could be detached by forceps without damaging the mouthparts. A maximum time interval of 2 hours elapsed between removal of the parasitic stages of the tick from one host, and replacement on another.

The patches on the recipients were cut open 48 hours after the transfer operation and the shaven area searched for any engorging ticks. Just before the tick moult, the host animal was thoroughly sprayed with Dursban 0.05% to ensure that all ticks were killed.

(c) TRANSMISSION BY TRANS-STADIAL TRANSFER. A procedure similar to that used in the intrastadial transfers was followed but the parasitic stages were allowed to engorge before collection. After moulting in the constant temperature

and humidity room, the newly emerged stages were checked and sorted microscopically before being used to infest the recipient host. Transfer stages were allowed to attach to the susceptible host for 3 days before being sprayed with Dursban 0.028%. The time off a host varied from 16 to 18 hours.

#### IV. RESULTS

Anaplasmosis was not detected in any calves during the period from splenectomy to the commencement of the experiments.

(a) TRANSMISSION BY TRANSOVARIAL METHODS. Four groups of experiments were performed in this trial—

EXPERIMENT 1. Steers (no. 1, 2, 3 and 4) had parasitaemias of 6%, 22%, 24% and 32% respectively when parent ticks were engorging.

EXPERIMENT 2. In this experiment, inoculation was arranged so that the larval, nymphal and adult stages were feeding during a period of up to 50% parasitaemia.

EXPERIMENT 3. A field strain inoculum containing *A. marginale* was used to produce a parasitaemia of 3% in the donor calf. This strain was used in case the laboratory strain had lost its ability to cycle through the ticks.

EXPERIMENT 4. Again the same basic procedure was followed, but the larvae used to infest the susceptible recipient were the progeny of replete field strain ticks that had engorged on an animal naturally infected.

*A. marginale* was not observed in blood smears from any of the recipient calves infested with larval progeny of adult female *B. microplus* engorging on donor calves infected with *Anaplasma*.

No *Anaplasma* CF antibodies were detected in weekly serum samples taken from the recipients during the 90 days after infestation. On challenge with *A. marginale* stabilate, all calves showed *A. marginale* parasitaemias in excess of 1%, 26 to 34 days after inoculation.

(b) TRANSMISSION BY INTRASTADIAL METHODS. Table 1 summarizes the results obtained. Transmission was successful with all stages during their mid-instar transfer.

(c) TRANSMISSION BY TRANS-STADIAL METHODS. Transmission was successful with all newly moulted stages (table 2) except for the first attempt at nymph-to-adult female transmission. This experiment was repeated and transmission was achieved.

#### V. DISCUSSION

The design of these experiments and the interpretation of the results were based on the fact that *B. microplus* is a one-host tick. Hitchcock (1955) investigated the life cycle of this tick on its bovine host and reported that the duration of the larval stage on the host varied between 4.5 days and 13.2 days. Therefore tick transfers at 2.5 days after infestation would have been by larval stages only.

Positive diagnosis of anaplasmosis from blood films stained by the Giemsa-staining procedure was difficult even for experienced workers, especially where the percentage of infected cells was low and where dye deposits may have been confused with the parasite. Infection in at least 0.5% of red blood cells was taken as a standard to guard against an error in diagnosis.

TABLE 1  
TRANSMISSION BY INTRASTADIAL TRANSFER

Experiment Number	Stage Transferred	Age of Ticks when Transferred (days)	Number of Ticks Transferred	Donor Host Parasitaemia (%)	Period from Stage Transfer to 0.5% <i>A. marginale</i> Parasitaemia (days)
1 Interrupted feeding of larvae ..	larvae .. ..	2-5	26 larvae	52—A.R.I. strain	28
2 Interrupted feeding of nymphs	nymphs .. ..	5-7	30 nymphs	8—Wacol strain	27
3 Interrupted feeding of adults ..	adults ..	male ..	22 male adults	8—Wacol strain	28
		female ..	20 female adults	24—Wacol strain	27

TABLE 2  
TRANSMISSION BY TRANS-STADIAL TRANSFER

Experiment Number	Stage Transferred	Number of Ticks Transferred	Host Parasitaemia (%)	Period from Stage Transfer to 0.5% <i>A. marginale</i> Parasitaemia (days)
1 .. ..	A .. .. larvae-to-nymph	36 nymphs	50—A.R.I. strain	44
	B .. .. larvae-to-nymph	41 nymphs	27—A.R.I. strain	42
2 .. ..	nymph-to-adult male	10 male adults	10—A.R.I. strain	47
	nymph-to-adult female	34 female adults	12—A.R.I. strain	43

Mechanical transmission has been demonstrated with horseflies (Howell 1957). Large numbers of these flies were required to effect a transfer from infected to susceptible cattle within minutes of taking blood. In contrast, our intrastadial transfers (perhaps mechanical transfers) were successful using smaller numbers of ticks and longer time intervals off the hosts, that is, up to 2 hours. These time intervals ensured that fresh blood did not remain on the mouthparts. Extra-erythrocytic initial *Anaplasma* bodies ingested with the plasma during larval feeding, and infected whole blood taken by the nymphal and adult stages, may have been retained until later regurgitated when feeding resumed on the susceptible host. A multiplication phase could have existed in the tick gut.

It was thought desirable to complete the trans-stadial transfers as quickly as possible. A previous experiment of this kind, in which the time off the host was 11 days, did not result in transmission (Connell, unpublished 1971). However, Bram (1971) using the three-host tick *Dermacentor andersoni* Stiles was successful with trans-stadial experiments in which the times off the hosts ranged from 27 to 65 days. If the *Anaplasma* body survived the digestive processes and did not degenerate, then the reorganization of the tick tissues during the moult may have enabled the parasite, in perhaps a different form from that in bovine blood, to reach the salivary glands. After the moult, the act of feeding could have initiated the final development of the parasite before being passed to the bovine host. Bram and Romanowski (1970) fed nymphs of *D. andersoni* on calves infected with *Anaplasma*. Using the fluorescent antibody method they were able to identify *A. marginale* in smears prepared from gut contents of nymphs for 24 hr after detachment. However, they failed to confirm reports that *Anaplasma* could be recognized in salivary glands or Malpighian tubules, or that multiplication by binary fission occurred in these tubules. A blood sample, held at 80% R.H. and 32°C, was used as a control for these experiments and exhibited specific fluorescence of *Anaplasma* for 5 days.

For infection to pass to the progeny, there must be a multiplication of the parasite in the body of the adult tick, otherwise the dilution of the original quantity of organisms as they pass from the female to subsequent generations would be so great that the infection would be lost. Apparently infection did not persist long enough in the adults to be transmitted transovarially.

The incubation periods (that is, the intervals between inoculation with *A. marginale* or infestation by ticks carrying *Anaplasma* and the first appearance of parasites in Giemsa-stained films) obtained for our work varied between 26 and 47 days. Generally, incubation periods produced in recipient hosts by intrastadial transfer of ticks were consistent with those produced by blood inoculation of the original hosts. This was true for the three *Anaplasma* strains employed. Franklin, Heck and Huff (1963) discussed incubation periods in relation to volume of inoculum, infectivity of inoculum, and environmental temperature of the recipient—an increase in all three could reduce the incubation period, whereas a decrease in just one factor could extend it. The A.R.I. strain generally produced incubation periods of 40 days or more, even though experiments using this strain of *Anaplasma* were conducted during the summer months. However, a repeat of the intrastadial transfer of larvae trial, again using the A.R.I. strain of *A. marginale*, resulted in an incubation period of 47 days, after which parasites developed in fewer than 1% of the bovine erythrocytes. If this long incubation period could be explained by the operation of an interferon system, then results would indicate that trans-stadial transmissions produced longer incubation periods than intrastadial transmissions. Marble and Hanks (1972) postulated a developmental cycle of *A. marginale* in the precursor cell

to the erythrocyte before the *Anaplasma* invaded the red blood cells during acute infection. Only one strain, the A.R.I. strain of *A. marginale*, was utilized in the trans-stadial trials.

Incubation periods in these experiments were consistent with those reported in the literature either for mechanical transmission by biting flies or stadial transfer by three-host ticks (Rees 1934; Anthony and Roby 1966; Bram 1971).

Anaplasmosis transmission has been demonstrated experimentally by intrastadial and trans-stadial transfer, but the importance of these methods in the natural transmission of the disease is not known. Numerous field and laboratory reports describe *B. microplus* movement on cattle (Regendanz 1933; Bennett and Wharton 1968; Uilenberg 1970; Roberts 1971; R. W. Sutherst, personal communication 1973). Seddon and Albiston (1966, Part 3) discussed the ability of larvae actively or passively to migrate on the ground.

This movement may be sufficient to continue infections with *A. marginale* in Australia without requiring a transovarial method of transfer.

## VI. ACKNOWLEDGEMENT

The author wishes to thank Mr. W. T. K. Hall (Director of Pathology) for advice in planning the work and assistance with the manuscript. The assistance given by Mr. C. C. Roberts is also acknowledged.

## REFERENCES

- ANTHONY, D. W., and ROBY, T. O. (1966).—The experimental transmission of bovine anaplasmosis by three species of North American ticks. *Am. J. vet. Res.* 27:191-198.
- BENNETT, G. F., and WHARTON, R. H. (1968).—Variability of host resistance to cattle tick. *Proc. ecol. Soc. Aust.* 3:150-154.
- BRAM, R. A. (1971).—Trans-stadial (Nymph-to-Adult) infection of *Anaplasma marginale* in *Dermacentor andersoni* Stiles. *J. med. Ent.* 8:519-521.
- BRAM, R. A., and ROMANOWESKI, R. D. (1970).—Recognition of *Anaplasma marginale* Theiler in *Dermacentor andersoni* Stiles. (= *D. venustus* Marx.) by the Fluorescent Antibody method. I. Smears of nymphal organs. *J. Parasit.* 56:32-38.
- BRUMPT, E. (1920).—Les piroplasmes des bovidés et leurs hôtes vecteurs. *Bull. Soc. Path. exot.* 13:416-460.
- BRUMPT, E. (1931).—Transmission *D'Anaplasma marginale* par *Rhipicephalus bursa* et par *Margaropus*. *Ann. Parasit. hum. comp.* 9:4-10.
- CALLOW, L. L., and HOYTE, H. M. D. (1961).—Transmission experiments using *Babesia bigemina*, *Theileria mutans*, *Borrelia* sp., and the cattle tick, *Boophilus microplus*. *Aust. vet. J.* 37:381-390.
- CONNELL, M. L., and HALL, W. T. K. (1972).—Transmission of *Anaplasma marginale* by the cattle tick, *Boophilus microplus*. *Aust. vet. J.* 48:477.
- DE FARIA, J. G. (1928).—Estudios sobre la tristeza de los bovinos. Proplasmosis y anaplasmosis. *Revta. Inst. bact.*, B. Aires. 5:429-469.
- DIKMANS, G. (1950).—The transmission of anaplasmosis. *Am. J. vet. Res.* 11:5-16.
- FRANKLIN, T. E., HECK, F. C., and HUFF, J. W. (1963).—Anaplasmosis Complement—Fixation Antigen Production. *Am. J. vet. Res.* 24:483-487.
- HITCHCOCK, L. F. (1955).—Studies of the parasitic stages of the cattle tick, *Boophilus microplus* (Canestrini.) (Acarina: Ixodidae). *Aust. J. Zool.* 3:145-155.

- HOWELL, D. E. (1957).—Anaplasmosis in cattle. *Proc. 3rd Natn. Res. Conf.*, Manhattan, Kansas, p. 14.
- LEATCH, G. (1973).—Preliminary Studies on the transmission of *Anaplasma marginale* by *Boophilus microplus*. *Aust. vet. J.* 49:16-19.
- LEGG, J. (1933).—The occurrence of *Anaplasma marginale* Theiler, 1910 in northern Queensland. *Pamph. Coun. scient. ind. Res. Aust.* No. 38 p. 31.
- LIGNIÈRES, J. (1919).—Piroplasmes, anaplasmes et grains chromatique. *Bull. Soc. Path. exot.* 12:558-556.
- MACKERRAS, I. M., MACKERRAS, M. J., and MULHEARN, C. R. (1942).—Attempted transmission of *Anaplasma marginale* Theiler by biting flies. *J. Coun. scient. ind. Res. Aust.* 15:1-18.
- MARBLE, D. W., and HANKS, M. A. (1972).—A tissue culture method for *Anaplasma marginale*. *Cornell Vet.* 62:196-205.
- MARSHALL, J. D., EVELAND, W. C., and SMITH, C. W. (1958).—Superiority of Fluorescein Isothiocyanate (Riggs) for Fluorescent-Antibody technique with a modification of its application. *Proc. Soc. exp. Biol. Med.* 98:898-900.
- NEITZ, W. O. (1956).—A consolidation of our knowledge of the transmission of tick-borne diseases. *Onderstepoort J. vet. Res.* 27:115-163.
- PHILIP, C. B. (1963).—Ticks as purveyors of animal ailments: a review of personal data and recent contributions. *Adv. Acarol.* 1:285-325.
- PIERCY, P. L. (1956).—Transmission of anaplasmosis. *Ann. N.Y. Acad. Sci.* 64:Art 2:40-48.
- PRICE, K. E., POELMA, L. J., and FABER, J. E. (1952).—Preparation of an improved antigen for anaplasmosis Complement-Fixation tests. *Am. J. vet. Res.* 13:149-156.
- QUEVEDO, J. M. (1916).—"Transmission natural de la anaplasmosis bovins. Buenos Aires" p. 4 Cited by Rees, C. W. (1934). *Tech. Bull. U. S. Dep. Agric.* No. 418 p. 17.
- QUEVEDO, J. M. (1929).—"La "Tristeza" de los Bovines (Piroplasmosis—Babesielosis—Anaplasmosis). Buenos Aires" p. 196. Cited by Rees, C. W. (1934). *Tech. Bull. U.S. Dep. Agric.* No. 418 p. 17.
- REES, C. W. (1934).—Transmission of anaplasmosis by various species of ticks. *Tech. Bull. U.S. Dep. Agric.* 418:1-17.
- REGENDANZ, P. (1933).—Die Übertragung von *Anaplasma* durch *Boophilus microplus*. *Zentbl. Bakt. ParasitKde Originale* 130:214-220.
- RISTIC, M. (1962).—A Capillary Tube Agglutination test for anaplasmosis.—A preliminary Report. *J. Am. vet. med. Ass.* 141:588-594.
- RISTIC, M. (1968).—"Infectious Blood Diseases of Man and Animals", Vol. II. Academic Press, Inc.; New York, p. 473.
- RISTIC, M., and WATRACH, A. M. (1963).—Anaplasmosis. VI. Studies and a hypothesis concerning the cycle of development of the causative agent. *Am. J. vet. Res.* 24:267-277.
- ROBERTS, J. A. (1971).—Behaviour of larvae of the cattle tick, *Boophilus microplus* (Canestrini), on cattle of differing degrees of resistance. *J. Parasit.* 57:651-656.
- ROGERS, R. J. (1971).—Bovine anaplasmosis: An evaluation of the Complement-Fixation and Capillary Tube-Agglutination tests and the incidence of antibodies in northern Queensland cattle herds. *Aust. vet. J.* 47:364-369.
- ROSENBUSCH, F. and GONZALEZ, R. (1927).—Die Tristeza—Übertragung durch Zecken und dessen Immunitats probleme. Experimentelle Untersuchungen (II. Mitteilung). *Arch. Protistenk.* 58:300-320.
- SEDDON, H. R. (1966a).—"Diseases of domestic animals in Australia—Part 3, Arthropod infestation (ticks and mites)." Commonw. Aust. Dep. Hlth Serv. Publ. No. 8 (Second Ed. revised by H. E. Albiston), p. 7.
- SEDDON, H. R. (1966b).—"Diseases of domestic animals in Australia—Part 4, Protozan and virus diseases." Commonw. Aust. Dep. Hlth Serv. Publ. No. 8 (Second Ed. revised by H. E. Albiston), p. 3.
- SERGEANT, E., DONATIEN, A., PARROT, L., et LESTOQUARD, F. (1945).—"Etudes sur les piroplasmoses bovines". Institut. Pasteur d'Algerie, Alger. pp. 636-637.
- THE, T. H., and FELTKAMP, T. E. W. (1970).—Conjugation of Fluorescein Isothiocyanate to antibodies. II. A Reproducible Method. *Immunology* 18:875-881.



- THEILER, A. (1912).—Übertragung der anaplasmosis mittels Zecken. *Z. InfektKrankh. parasit. Krankh. Hyg. Haustiere.* 12:105-116.
- UILENBERG, G. (1968).—Notes sur les babesioses et l'anaplasmosose des bovins à Madagascar. I. Introduction—Transmission. *Revue Elev. Méd. vét. Pays trop.* 21:467-474.
- UILENBERG, G. (1970).—Notes sur les babesioses et l'anaplasmosose des bovins à Madagascar. IV. Note additionnelle sur la transmission. *Revue Elev. Méd. vét. Pays trop.* 23:309-312.

(Received for publication 2 April 1974)

The author is parasitologist in the Pathology Branch, Animal Research Institute, Queensland Department of Primary Industries, stationed at Yeerongpilly.