Name: Mario Perez Date of Degree: May 14, 1972

Institution: Oklahoma State University Location: Stillwater, Oklahoma

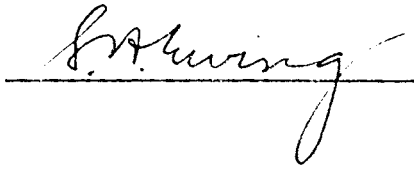
Title of Study: A REVIEW OF ANAPLASMOSIS

Pages in Study: 58 Candidate for Degree of Master of Science

Major Field: Veterinary Parasitology

Scope of Study: In this report an attempt is made to summarize the most significant conclusions from reported research dealing with anaplasmosis and with the etiologic agent of this disease, Anaplasma marginale. The organism and the disease it causes are described and an attempt made to identify areas of application for the research, especially in developing countries such as Colombia.

Findings and Conclusions: Although Anaplasma marginale has been studied extensively, information is still lacking concerning certain features of its biology. It is well known that the initiator of infection is the initial Anaplasma body. Reproduction of the organism is still controversial since binary fission and budding have been postulated. Ticks and horseflies are the most important animate vectors of the disease, and cattle which have recovered from illness are the most important reservoir hosts. Carriers can be freed of infection by tetracycline therapy, but they may become reinfected making this practice an unsuitable solution to control of the disease in Colombia. Presently available vaccines prevent clinical manifestations of the disease and reduce death losses but do not prevent anaplasmosis infection. Inoculation of Anaplasma organisms into young calves produces a state of premunition and could be a practical and economical method to reduce losses due to the disease in Colombia. This practice cannot be used in well developed countries and has serious shortcomings; however, it might be recommended for use in Colombia because of its geographical location where animate vectors are active the entire year.

ADVISER'S APPROVAL 

A REVIEW OF ANAPLASMOSIS

By

MARIO PEREZ

Doctor of Veterinary Medicine

Universidad Nacional

Bogota, Colombia

1967

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 1972

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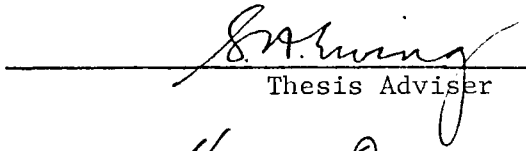
Report Approved:



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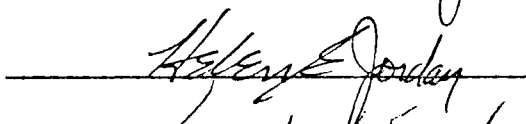

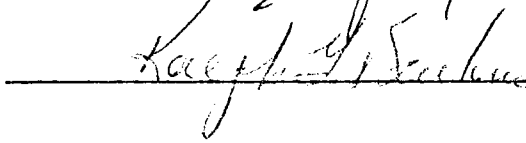

Dean of the Graduate College

ACKNOWLEDGEMENTS

The writer expresses sincere gratitude to Doctor S. A. Ewing, his major adviser, and Head of the Department of Veterinary Parasitology and Public Health for his guidance and assistance.

Thanks are extended to Doctor H. E. Jordan and Doctor R. G. Buckner, members of the Committee for their helpful criticisms and suggestions.

Special thanks are due to Doctor T. R. Thedford and Doctor P. B. Barto for preliminary corrections of this manuscript.

The writer is indebted to Mrs. B. Williams, Mrs. L. Hamblin, and Mrs. B. Miller for their assistance in typing this manuscript.

Special appreciation is due my wife, Sonia, for her patience and encouragement.

Appreciation is also expressed to the Agency for International Development (AID) for providing financial support.

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CHAPTER I

INTRODUCTION

Cattle provide an important source of animal protein for man through their meat and milk. Successful production of livestock is influenced by many factors including animal diseases which adversely affect growth and reproductive efficiency. Among the important diseases are many which are infectious, and one of these is anaplasmosis. This disease is caused by a microorganism termed Anaplasma marginale which was discovered about 60 years ago.

Anaplasmosis is a disease of great economic importance to the cattle producers of the world, plaguing both the dairy and beef cattle industries. Cattle owners are greatly concerned about its prevention and control.

According to Welter and Woods (1968), losses due to anaplasmosis in the United States have been estimated to be approximately 34 to 35 million dollars annually. Losses are also significant in other parts of the world. Losses caused by the disease are not restricted to mortality, but also result from a high morbidity which results in weight loss, early weaning, orphaned calves and decreased milk production. In addition, considerable money is spent for drugs, veterinary medical fees, and diagnostic services (Garlick, 1967).

The disease is rather unusual in that carrier animals remain permanently infected. These serve as a source of infection for susceptible

animals.

Tropical countries like those of Latin America which have pasture all year round, provide abundant and economically important grazing lands. These countries could improve and accelerate their development through a highly efficient livestock industry. In an attempt to improve their livestock, these countries import purebred cattle from other countries. Unfortunately, attempts at introduction of these cattle to the tropical areas usually meet with failure as a result of losses due to diseases. A great percentage of the investment made by many cattlemen has been lost due to outbreaks of anaplasmosis.

The purpose of this paper is to review the most important research which has been done on anaplasmosis. Based on available literature it appears that almost all efforts of the investigators have been devoted to the study of A. marginale. This may be due to the fact that this organism is more important economically than the related organisms such as Anaplasma centrale, Anaplasma ovis, Paranaplasma discoides and Paranaplasma caudata. Therefore, in this review, emphasis will be given to Anaplasma marginale although organisms related to it will be mentioned when needed throughout this review.

It is hoped that this collation of literature will be helpful to my country (Colombia), to the students in the field of veterinary medicine and to my colleagues. Modern scientific literature is not readily available to many of the developing countries in Latin America and this paper will help to fill a void concerning a very important disease.

CHAPTER II

REVIEW OF LITERATURE

A Review of Anaplasmosis

Definition

Anaplasma marginale is an entity which produces or is associated with the disease known as anaplasmosis in cattle (Krull, 1969). The disease is also sometimes called gall-sickness (Lapage, 1968).

In cattle severe debility, emaciation, anemia and jaundice are the major clinical signs. The disease is usually sub-clinical in sheep and goats (Blood and Henderson, 1963).

Classification

Although once considered to be a protozoan there is little doubt that *Anaplasma* should be regarded as rickettsial in nature (Breed, Murray and Smith, 1957). The structural and developmental resemblance between the so-called initial *Anaplasma* body and certain well-known rickettsiae along with its close morphologic, serologic, and metabolic resemblance to species in the genera *Eperythrozoon* and *Haemobartonella* have contributed to classification of the genus *Anaplasma* in the Order Rickettsiales (Blood and Henderson, 1963).

As previously stated, the organism generally is considered to be a rickettsia but according to Lapage (1968), *Anaplasma* has also been

included in the Order Haemosporidia, and the following species are described:

Anaplasma marginale Theiler, 1910

Anaplasma centrale Theiler, 1911

Anaplasma ovis Lestoquard, 1924

According to Monnig (1968), A. ovis is referred to in the literature occasionally, but there is some doubt as to the validity of the species. The names Paranaplasma discoides and Paranaplasma caudata have been given by Kreier and Ristic (1963a and 1963b) to organisms which have been found associated with A. marginale in the Oregon strain of Anaplasma. The taxonomic validity of these species is yet to be determined.

Description of Related Organisms

A. centrale. Waddel (1964) studied this organism by means of electron microscopy. He found that the fine structure of A. centrale was very similar to that of A. marginale. Anaplasma centrale was composed of one to at least six subunits enclosed within a double membrane. The subunits varied in size from 250 mu to 400 mu in diameter and had a double membrane. In some organisms containing three or more subunits, blunt projections of the limiting membrane up to 70 mu long extended into the erythrocyte substance. In Anaplasma centrale the majority of Anaplasma bodies are located toward the center of the cell, away from the periphery. It is erroneous, however, to assume that all the organisms found in A. centrale infection are located in the center and those in A. marginale infections are found only in the cell margins. Considerable variation in location does occur (Kuttler, 1966). Apparently very

little is known concerning the morphology of A. centrale.

Anaplasma ovis. This organism is the causative agent of anaplasmosis in sheep and goats. As previously stated, there is some doubt that this is a valid species, but no one has yet shown conclusively that it is not. In a recent study of this organism by means of electron microscopy, Jatkar (1969) concluded that the membrane-enclosed body was usually marginally located and most of the marginal bodies observed were single. A single-walled limiting membrane and two initial bodies were observed. The two initial bodies completely filled the marginal body. Both initial bodies were enclosed by a double-layered membrane. Material in the initial bodies was coarse and filamentous.

Paranaplasma discoides and Paranaplasma caudata. Kreier and Ristic (1963a) studied water-lysed erythrocytes from calves acutely infected with the so-called Oregon strain of Anaplasma. They observed by phase microscopy that some of the organisms had an ovoid disc-like structure with two denser masses, one at each pole. The structure was completely inside the erythrocytic stroma. These workers proposed the name Paranaplasma discoides for this organism. Later Kreier and Ristic (1963b) stated that the Oregon strain consisted of a mixed infection of three organisms, viz., the round marginal body type, the traditional A. marginale, the bipolar disc type, P. discoides, and finally an organism possessing a head, body and tail which they referred to as Paranaplasma caudata. Paranaplasma caudata is visible only by special techniques. These workers observed that sheep passage of the Oregon strain eliminated P. caudata but permitted the survival of A. marginale and P. discoides.

Boynton (1932, cited by Weinman and Ristic, 1968) was the first who described tailed parasites in films stained with Toisson's fluid from cattle with acute anaplasmosis. Franklin and Redmond (1958) observed projections, or tails, extending from otherwise typical Anaplasma bodies. The authors were of the opinion that this phenomenon represented one stage in the normal development of A. marginale in the blood of cattle.

Earlier, Pilcher, Wu, and Muth (1961) studied a strain of Anaplasma from Oregon. They lysed the host cells and studied the organism by phase-contrast microscope. The form most frequently observed possessed two parts, one of which was more or less spherical and was referred to as the head portion. Attached to the head was a tail-like appendage. Using the Giemsa stain only typical marginal Anaplasma bodies were found.

Madden (1962) studied A. marginale using the Oregon strain by means of fluorescein-labeled antibody technique. About 70% of the forms were composed of a round head and a tail. The anaplasmata appeared as comma, comet, and matchstick forms. Another form observed by this worker resembled a dumbbell, having two round heads connected by a tail. According to this author the tail is an integral part of the organism, since the fluorescent antibody technique embodies the specificity of the antigen-antibody reaction.

Ritchie (1962) studied A. marginale in dehemoglobinized erythrocytes using electron microscopy. Blood samples were taken from splenectomized calves in the acute phase of the disease. He observed that the most prominent characteristics of the parasite were a terminal round electron-dense structure and an associated sac-like projection. This type of

parasite was quite similar if not identical to that later named Paranaplasma caudata by Kreier and Ristic (1963b).

Simpson, Kling, and Neal (1965) acknowledged that isolates of Anaplasma from Mexico, California and Óregon had been reported to differ from other strains by having projections that extended from the inclusion. These structures which were not visible by Giemsa staining had been described variously as an integral part of the marginal inclusion, as a flagellate, as an artifact, and as an unknown parasite. Simpson, Kling, and Neal (1965), using light microscopy, studied marginal inclusions with elongated comet and dumbbell-shaped projections in unfixed smears stained with Goodpasture's, Noland's, and new methylene blue. The same material was also observed in thin sections by electron microscopy. They found that blood fixed in OsO_4 contained projections which were elongated, straight, and had a rigid appearance. Sometimes the projections were forked at the free extremity. It was concluded from this study that the projections might have been formed from organization of proteinaceous material in parasitized erythrocytes.

Kreier and Ristic (1963a), working with the Oregon strain of Anaplasma from water-lysed erythrocytes, observed organisms with a long tail which extended all the way across the cell. The head of these organisms, which was sometimes single, sometimes double, was often outside the erythrocytic stroma. Typical round bodies and others shaped like bipolar discs were also observed.

Kreier and Ristic (1963c) studied the Oregon strain of Anaplasma stained with labeled antibody specific for that strain. They found besides the typical round marginal bodies organisms with a round or oval head, a body, and a long tail. They concluded that since the body and

the tail of the parasite can be stained with fluorescein-labeled antibody these structures are a portion of the parasite. It was also demonstrated in this study that both marginal body types of organisms found in the Oregon and in the Florida strains of Anaplasma were antigenically identical but the parasite characterized by a head, body and tail found in the Oregon strain was antigenically and immunoserologically distinct to both marginal body types.

Geographic Distribution

Anaplasma marginale. This organism is widely distributed throughout the tropical and sub-tropical areas of the world. It is found in cattle; and it is common in Africa, the Middle East, Southern Europe, and the Far East (Monnig, 1968), and in the United States, except possibly in Alaska, Wisconsin, Maine, Vermont, New Hampshire, Massachusetts, Connecticut, and Rhode Island. It was also present in the state of Hawaii until recently. It is endemic from Maryland down the Atlantic Coastal States to Florida, from which it continues in the Coastal States to Mexico. Parts of Kansas, Oklahoma, Missouri, Illinois, Kentucky, and Tennessee are included in the endemic area (Krull, 1969).

Anaplasma centrale is found in cattle in Africa, the Middle and Far East and Southern Europe (Lapage, 1968).

Anaplasma ovis occurs in sheep in North Africa, Palestine and South Africa (Lapage, 1968). It also has been reported from North America and the Soviet Union (Monnig, 1968).

Host Range

Invertebrate. Anthony, Madden and Gates (1964) have stated that the

behavior of A. marginale in its vectors is still not understood. Friedhoff and Ristic (1966) said that attempts to detect Anaplasma organisms in the tissues of vector ticks have not been successful.

Anaplasmata were found by immunofluorescent methods in smear preparations of gut and excreta from Dermacentor andersoni specimens that had fed on infected calves. Anaplasmata were found by brightfield techniques and by electron microscopy in excreta preparations. Electron microscopic examination of ultrathin sections through gut deverticula, revealed structures, believed to be A. marginale in undigested erythrocytes, but anaplasmata were not found in ultrathin sections of the salivary glands and reproductive organs (Anthony, Madden and Gates, 1964).

Friedhoff and Ristic (1966) demonstrated A. marginale in the gut contents and in the Malpighian tubules of engorged Dermacentor andersoni nymphs by the fluorescent-antibody technique. There was evidence that Anaplasma organisms multiplied in the Malpighian tubules by the process of binary fission. Anaplasma marginale bodies were found in the gut contents of the nymphs on the day of detachment. Specifically stained fluorescing bodies were found in the cells of the Malpighian tubules of nymphs on the 1st, 3rd, 4th, and 5th days after attachment. These authors found no indication of Anaplasma multiplication in either Dermacentor variabilis or D. albipictus following the ingestion of infected bovine blood.

Vertebrate. Anaplasma marginale. All breeds of cattle apparently are susceptible but some individual Brahman cross-breeds seem to show a partial resistance. The other mammals that have been reported to be susceptible to infection include the pig, antelope, elk, buffalo, camel, sheep, and goat, but more experimental work needs to be done to determine

their exact status. On the basis of recent work with sheep and goats, there is reason to believe that these are not hosts (Krull, 1969). In 1933, Boynton and Woods transferred A. marginale from cattle to deer and back to cattle using a southern black-tailed deer (Odocoileus hemionus hemionus). They also infected a cow with A. marginale using a mixture of blood from seven wild Columbian black-tailed deer (O. hemionus columbianus). Christensen, Osebold and Rosen (1958), Christensen, et al., (1960), Osebold, Douglas, and Christensen (1962), and Howe, et al., (1964) have also reported anaplasmosis in deer. Bedell and Miller (1966) reported that there was no evidence of anaplasmosis in 262 white-tailed deer in nine southeastern states; they used Giemsa stained smears and inoculation of blood into splenectomized calves as diagnostic tools.

Anaplasma centrale is found in cattle and A. ovis is found in sheep and goats. Some experts consider that A. centrale is a variety of A. marginale and it seems possible that the forms occurring in sheep and goats, to which the name A. ovis has been given, may also be a variety of A. marginale (Lapage, 1968).

Morphologic Features

Anaplasma appears as small, spherical bodies, red to dark purple in color when stained with Romanowsky stains, inside the red blood cells of cattle, deer, sheep and goats. They are 0.2 u - 0.5 u in diameter, with no cytoplasm, but a faint halo may appear around them. Sometimes two organisms may lie close to each other, giving the appearance of binary fission; occasionally multiple invasion of a cell may occur (Monnig, 1968). Many other shapes including rod, comma, ring, triangular, rough, smooth, and sporoid have been described by workers using light microscopy and

different methods of preparation (Brock, 1962).

In smears stained with Wright-Giemsa, A. marginale appears as compact, deeply stained, punctiform bodies of from 0.2 to 1.2 u in diameter, generally located in a marginal position in the erythrocyte (Espana and Espana, 1962).

Ristic and Kreier (1963) state that with the acridine orange (AO) staining method, it was shown that the marginal body was not a homogenous single unit, but rather an agglomeration containing several subunits known as initial bodies.

When viewed by phase microscopy in hemolyzed erythrocytes the organism appears either: (1) as round, oval or slightly irregularly shaped bodies, similar to those seen in stained smears, the larger ones often seeming to consist of several compact globular units or (2) as apparently identical structures to which are attached filaments. When the anaplasmatas turn, some of the filaments may appear less compact and resemble comets, suggesting that the structure might be a membrane instead of a filament (Espana and Espana, 1962). These workers also found parasites in which the filament was in the form of a ring.

The filamentous structures have also been demonstrated in preparations stained with modifications of Fontana's silver impregnation and Noland's stain for ciliates (Espana and Espana, 1962). According to Ristic (1967) attempts have been made to define the morphologic features of the Anaplasma body by means of a variety of techniques. Some of the techniques were staining of infected blood with fluro-chrome dyes, methyl green pyronine and periodic acid-Schiff methods, the fluorescent antibody methods, electron microscopy of ultra thin sections, and the shadow-cast preparations of the infected erythrocytes. Studies with

these techniques indicated that the Anaplasma body consisted of 1 to 8 initial bodies embedded in a homogenous matrix. Single, intraerythrocytic initial bodies, which apparently represent the infective form of Anaplasma, were enclosed in a double membrane, were round to oval and measured 0.3 to 0.4 u in diameter. These bodies have an outer narrow matrix that is demarcated from the cytoplasm of the erythrocyte by a membrane similar to that surrounding the marginal body. It is not known whether the outer matrix substance of Anaplasma is a component of the host cell, an integral part of the parasite, or a product of both the parasite and the host cell.

Ristic (1967) studied initial Anaplasma bodies free from erythrocytes; they were stained by the negative contrast technique and examined by electron microscopy. With this negative-contrast staining technique he revealed certain structural features of the initial Anaplasma bodies. The outer envelope possessed by erythrocyte-free initial bodies can be considered an integral component of the organism rather than of the erythrocytes. It was shown in this study that the envelope of the initial body gradually develops into a matrix by reproduction of the initial body at the time that formation of a marginal body has begun. When the marginal body (an inclusion of 2 or more initial bodies) has been formed, the envelope becomes the matrix embedding the marginal body and the newly formed initial bodies have a common envelope structure. At this stage of development of Anaplasma, the envelope of the initial body apparently becomes a matrix of the marginal body.

Simpson, Kling and Love (1967) presented new information about A. marginale as derived from electron microscopic studies. According to them one Anaplasma body (marginal body) contained from 1 to 6 subunits.

A subunit (initial body) varied between 0.3 and 0.6 u in diameter and was surrounded by a double membrane. The subunit was round when an Anaplasma body was composed of a single subunit. However, when the Anaplasma body contained more than 1 subunit, the subunits varied from bean-shaped to circular, with intermediate forms. The variation in shape seemed to result from the divisionary process that transformed a single subunit into 2 subunits. The internal components of subunits consists of fibrillar material and various numbers of electron-dense granules. Such granules seem to be nucleoproteins composed of both ribonucleic acid and deoxyribonucleic acid, but Ellender and Dimopoullos (1967), who reported isolation of DNA from purified Anaplasma bodies, could not isolate RNA. The subunit or subunits in an Anaplasma body were located in intracytoplasmic vesicles in the erythrocytic cytoplasm. Such vesicles had a single membrane which occasionally contained small pores. It seemed that a single subunit divided by binary fission to form 2 subunits. Fission seemed to result from infoldings of one side, or opposite sides, of the double surrounding membranes of subunits. Simpson, Kling, and Love (1967), also described a possible mechanism of penetration and exodus of Anaplasma bodies from parasitized erythrocytes. The organisms seemed to invade erythrocytes by a type of pinocytosis, which has been designated rhopheocytosis or erythrocytic vesiculation. The plasmalemma of most erythrocytes parasitized by A. marginale was covered by a filamentous external coat in which opaque particles (probably ferritin) were entrapped. In this process of rhopheocytosis there seemed to be invagination of the erythrocytic cytoplasmic membrane and pocketing of the parasite located next to the invaginated membrane and the associated organism, thus forming a parasite-containing vesicle

separated from the plasmalemma of the parasitized erythrocyte.

One possible mode of exodus of Anaplasma bodies from parasitized erythrocytes appeared to be rhopheocytosis in reverse order. In micrographs of the phenomenon, it seemed that subunits were expelled from a nonhemolyzed host cell without injuring the plasmalemma of the involved erythrocyte. Thus the phenomenon provided a means by which subunits could move through cell membranes without causing them to rupture.

A second mode of exodus of subunits from red blood cells seemed to operate when erythrocytes were lysed and became fragile as a result of extensive parasitemia. In this situation, the erythrocytic membrane and the limiting membranes of the Anaplasma body were fragmented resulting in release of free subunits into the surrounding serum.

Summers and Padgett (1970) studied the ultrastructure of the initial bodies of A. marginale using the negative contrast technique. In the study of the intact initial body they observed that non-dividing initial bodies are round or oval varying from 225 to 480 mu in diameter. Initial bodies were enclosed in a membrane well separated from the double-layered plasma membrane of the organism. Material enclosed in the plasma membrane was found in two states: in one, there was a granular appearance and in the other, the internal material was organized in the form of a long ribbon-like or tubular coiled structure. This was observed in both intra-erythrocytic and extra-erythrocytic initial bodies. The coiled configuration of material had not been previously described.

Reproduction

Ristic and Watrach (1963), using the fluorescent antibody technique, observed four developmental stages of the organism. Based upon frequency

of occurrence of Anaplasma and its location in the infected erythrocytes, they classified as follows: (a) early stage, consisting of initial bodies; (b) mixed population stage, consisting of marginal and initial bodies; (c) vigorous growth and transfer stage; and (d) massive multiplication stage with a predominance of marginal bodies.

Initial Anaplasma bodies were first observed by electron microscopy on the 5th day following infection. The first marginal Anaplasma bodies, composed of two or three initial bodies, were observed on the 10th day post infection. Later in the course of infection, marginal bodies consisted of eight to ten initial bodies. Furthermore, they stated that the initial body has the ability to reproduce by binary fission. An early stage of reproduction is represented by elongation of the initial body and slight constriction of its double membrane. The next stage is marked by further constriction of its double membrane. At this stage two daughter organisms are formed. These daughter forms still remain connected by a narrow band of plasma. In the terminal stage of reproduction, the two daughter organisms are completely separated by their double membranes but remain attached to each other. Separation of the two daughter forms follows; this represents the final step of reproduction, and the initial bodies then appear as single subunits within the matrix of the marginal body.

The formation of the mature inclusion (marginal body) represents only a phase in the developmental cycle of the initial body which itself occurs with frequency only during the acute and convalescent phases of infection. The initial Anaplasma body (the initiator of the infectious cycle) represents the form of the organism essential for its interepizootic survival. The Anaplasma agent is infectious in all stages and,

like bacteria and rickettsiae, apparently does not have a non-infectious period in its cycle.

The exact mode of reproduction and the sequence of the morphologic changes of A. marginale under natural conditions cannot be determined entirely until the life cycle of the organism within arthropod vectors is elucidated. Of particular importance would be information concerning the possible multiplication of the organism in certain species of ticks.

Studying reproduction of the initial body and the development of the marginal body, Summers and Paddgett (1970) found that the first evidence of replication of the initial body was a detached body or thickened area located within the outer membrane of the erythrocyte but still in contact with the plasma membrane of the mother cell at one end. At this stage of development, the initial body possesses two bud-like projections or daughter cells located at the opposite ends of the mother cell. During the process of replication, the daughter cells increase in size, develop a complete plasma membrane, remain within the outer membrane of the mother cell but show complete independence. Division and growth of these structures result in an elongate structure containing two initial bodies of about equal size. They eventually separate to become single initial bodies which comprise the marginal bodies, considered to be the terminal stage of development of A. marginale. At the time of release, the membrane of the erythrocyte is ruptured and the initial bodies escape as a group and later disperse. Based on this study it was indicated that replication of initial bodies may be by a process resembling budding rather than by binary fission.

Transmission

Only ticks and horseflies have been considered important animate vectors of anaplasmosis. By extensive work in many parts of the world, 7 genera and approximately 20 species of ticks have been shown to transmit anaplasmosis. The fact that transmission can be effected by a certain tick under experimental conditions does not necessarily mean that the tick is a vector in nature. At present, only two species of ticks now occurring in the United States appear to have sufficient experimental and epidemiological support to be considered important natural vectors of anaplasmosis, viz., Dermacentor andersoni and Dermacentor occidentalis. Certain species capable of transmitting infection under experimental conditions may be eliminated from consideration as significant natural vectors in the United States, viz., Argas persicus, Rhipicephalus sanguineus, Boophilus microplus. Other species such as Dermacentor variabilis, Dermacentor albipictus, and Ixodes scapularis remain under suspicion as vectors pending further investigation (Howell, 1968).

The role of certain ticks as experimental vectors of anaplasmosis is summarized in Table I.

Experimental and epidemiological evidence points to horseflies as the most significant insect vectors of anaplasmosis, and Tabanus abactor and Tabanus sulcifrons are the most efficient tabanid vectors (Safford, 1966).

Transmission by horseflies is effected only by mechanical means, i.e., the direct transfer of blood from infected to susceptible cattle. To effect transmission, transfer to a susceptible host must take place within a few minutes after the fly feeds on an infected animal, during the

TABLE I

SUMMARY OF EXPERIMENTAL TRANSMISSION
OF *ANAPLASMA MARGINALE* BY TICKS

Vector	Type of Transmission Demonstrated	References
Argas persicus	Transstadial	Howell, Stiles, and Moe (1943)
Boophilus annulatus	Transovarian	Rees (1934)
Boophilus microplus	Transovarian	Quevedo (1929, cited by Howell, 1968)
Dermacentor albipictus	Transstadial	Boynton, et al., (1936)
Dermacentor andersoni	Transstadial, Transovarian	Rees (1933), and Howell, Stiles, and Moe (1941a)
Dermacentor occidentalis	Transstadial, Transovarian	Boynton, et al., (1936)
Dermacentor variabilis	Transstadial	Anthony and Roby (1966)
Ixodes scapularis	Transstadial	Rees (1934)
Rhipicephalus sanguineus	Transstadial	Rees (1930)

short period that fresh blood remains on the mouth parts (Howell, 1957).

On experimental and epidemiological grounds other dipterans such as stable fly (Stomoxys), deer flies (Chrysops) and horn flies (Siphona) are potential vectors of anaplasmosis, but their actual importance as natural vectors is unknown (Howell, 1957). Mechanical transmission has been demonstrated experimentally with mosquitoes of the genus Psorophora, but mosquitoes are not considered nearly as significant as horseflies as vectors of anaplasmosis (Howell, Stiles, and Moe, 1941b).

In addition, mechanical transmission by blood sampling needles and other instruments used in surgical procedures such as dehorning and castration are very important in anaplasmosis transmission (Jones and Brock, 1966).

In Utero Experimental Transmission

Trueblood, Swift, and Bear (1971) reported that the bovine fetus is a susceptible host and capable of producing antibodies to A. marginale. Metastatic infection of the dam from the fetus was also observed. The experiment was accomplished by inoculating Anaplasma-infected blood into the peritoneal cavity of a 100-days old bovine fetus in utero by means of laparotomy. The fetus was removed from the cow by cesarean section 41 days after inoculation. The dam was positive to the presurgical complement-fixation test, but Anaplasma bodies were not found in blood smears. At the time of cesarean section 30% of the erythrocytes of the dam and 23% of the erythrocytes of the fetus were infected with Anaplasma bodies, and both reacted positively to the complement-fixation test. A susceptible calf inoculated with blood from the fetus developed signs of anaplasmosis.

Since maternal antibodies do not cross the bovine placental barrier, they concluded that the antibody titer found in the fetal blood was due to a fetal immunological response. Kuttler (1962) has stated that CF antibodies are transferred to calves through colostrum.

Preservation

According to Roby (1968) preservation and storage of infectious agents is important in order to have materials available for research investigation. In the past, the method of maintaining a source of Anaplasma has been to keep an infected carrier bovine in isolation.

Turner (1944) reported that citrated blood of a calf infected with A. centrale retained its infectivity for 254 days when quickly frozen and stored at -72°C to -80°C by means of solid carbon dioxide. Barnett (1964) reported that bovine blood containing A. marginale was still infective and fully virulent to cattle after storage at -79°C for 356 days. Glycerol at a concentration of 7% was added to the blood to protect the red blood cells from hemolysis during freezing and thawing. Love, Valentine and Scales (1967) reported that the infectivity of A. marginale in bovine erythrocytes frozen in 30% glycerin at -79°C was retained 173 days. Summers (1967) showed that Anaplasma-infected blood frozen in 8-10% glycerin remained infective for 406 days. Summers and Matsuoka (1970) reported that the infectivity and virulence of A. marginale was retained after storage at -70°C for 4 1/2 years. Heparinized, glycerinated infected bovine erythrocytes were used.

In all the studies concerning stored frozen blood containing Anaplasma, the basic procedure was a gradual reduction in temperature.

Hruska and Brock (1966) reported trials with Anaplasma-infected blood which had been stored in liquid nitrogen. The trials showed that protective additives were needed to prevent excessive hemolysis of the red blood cells and to maintain the infectivity of the parasites. They used as additives either (1) a 10% solution of dimethyl-sulfoxide, or (2) a sugar solution containing 5.0% glucose and 9.35% sucrose in once-distilled, demineralized water.

Virulence

According to Roby (1968), attempts have been made to alter either the virulence or the infectivity of A. marginale. In conducting this type of investigation it is important that a distinction be made between a modification in the actual virulence and a decrease in infectivity, for it has been shown that small amounts of infectious material can result in a mild clinical syndrome.

It has been reported by Bedell and Dimopoullos (1963) that Anaplasma-infected blood held at 17-18°C and treated with sonic energy for periods as long as 3 1/2 hours was still infective for calves.

Other studies conducted by Edds, et al., (1964) revealed that X-irradiation of Anaplasma- infected blood might produce a mutant that might protect cattle against field exposure. They reported that doses of X-irradiation greater than 60 kilorads, or Cobalt-60 irradiation, greater than 75 kilorads, are necessary to alter or inactivate A. marginale to reduce infectivity for susceptible animals. Simpson, Neal, and Edds (1964) found that gamma-irradiation from a Cobalt-60 source destroyed the capacity of the parasites to produce infection. Wallace and Dimopoullos (1965a) exposed a partially purified suspension of

Anaplasma organisms to a source of Cobalt-60 irradiation and found that 75,000 to 100,000 kilorads decreased the infectivity, and that 125,000 kilorads destroyed the infectivity of the preparation.

Kuttler (1966) reported that whereas an isolate of A. marginale of African origin was much more virulent than was A. centrale (the mild variety used in some countries for premunition), two isolates of A. marginale from the United States produced signs of infection that were not significantly more severe than those produced by A. centrale.

Biochemical Properties

According to Roby (1968) biochemical investigations are aimed at determining the chemical properties of the parasite to effect a better understanding of the organism, to determine its proper classification biologically, and to know more about how it produces disease. Moulton and Christensen (1955) showed by histochemical methods that Anaplasma sp. contain the nucleic acids RNA and DNA. More recently, Ellender and Dimopoullos (1967) reported success in isolating DNA from purified Anaplasma bodies, but their attempts to isolate RNA were unsuccessful.

According to Ristic, Mann, and Kodras (1963) a part of the chemical structure of the parasite is made up of lipoproteins. Soluble antigens prepared from Anaplasma-infected erythrocytes were used in this study and it was shown that these antigens were degraded by the enzymes pepsin and lipase. The most recent study concerning lipid material in the parasite was conducted by Wallace, Dommert and Dimopoullos (1967). They found in washed preparations of Anaplasma, relatively large amounts of phospholipids, and smaller amounts of sterols, free fatty acids, triglycerids and sterol esters. With the exception of nucleic acids, which are not

present in the mature, normal erythrocytes, there appears to be close similarity between the chemical properties of the Anaplasma and that of the contents of the host cell. The observations of Amerault and Roby (1967) suggested that the soluble Anaplasma antigens may be metabolic products which are produced by the parasite from the red blood cell.

Enzymatic Activity

The metabolic activities of A. marginale and several enzymes associated with the organism and those of the infected erythrocytes were studied by Dimopoullos (1968) and his research group.

Catalase. Extracts of erythrocytes from calves infected with A. marginale were found to contain increased catalase activity over that observed for preparations of normal erythrocytes. The increase in this activity appeared concurrently with an increase in the number of erythrocytes containing marginal bodies (Wallace and Dimopoullos, 1965b).

Lactate Dehydrogenase (L.D.H.). Low levels of LDH were detected in extracts of partially purified marginal bodies. It is believed that the organism utilized LDH for metabolic activity necessary for energy production (Darre, Wallace, and Dimopoullos, 1967).

Adenosinetriphosphatase (ATPase). The possiblity of enzyme activity in the marginal bodies per se was investigated as a result of finding increased ATPase activity in erythrocytes from calves infected with A. marginale. Both partially purified bodies and preparations of bodies purified by sucrose density gradient centrifugation were tested and were found to contain high activities of the enzyme. An increase in erythrocytic ATPase activity occurred as the infection progressed, and it was suggested that the increase was due wholly or partially to the

ATPase-containing marginal bodies in the cells. ATPase has been used as an expression of the active transport mechanism in a variety of cells including the erythrocyte. It seems apparent that A. marginale exerts a definite influence upon the metabolism of the erythrocytic membrane and that this influence is manifested in the energy-dependent system of the cell (Garon and Dimopoullos, 1967).

Acetylcholinesterase (AchE). According to Wallace (1967) acetylcholinesterase activity in bovine blood is mostly confined to the erythrocytic membrane. Studies of the level of this enzyme in bovine blood during anaplasmosis demonstrated a decrease in activity beginning immediately after inoculation of calves with A. marginale. Although a direct relationship was not observed between AchE activity and numbers of erythrocytes, packed cell volume, or osmotic fragility there was always a substantial decrease in AchE prior to changes in these values. If the organism is capable of affecting the membrane-bound AchE, this could be a controlling factor in its entrance into the erythrocyte.

Nucleic Acids. Although Moulton and Christensen (1955) reported isolation of DNA and RNA from Anaplasma organisms as previously stated, Ellender and Dimopoullos (1967) in a recent study demonstrated DNA in A. marginale but they could not isolate RNA from this organism.

Lipids

Fatty Acid Composition. According to Wallace, Dommert and Dimopoullos (1967), lipids have also been found in A. marginale. Phospholipids, sterols, free fatty acids, triglycerids and sterol esters were all detected in purified preparations of the organism. The phospholipid fraction made up the largest percentage of the total

lipids.

Electrophoretic Mobility. Dommert and Dimopoullos (1966) reported that the electrophoretic mobilities of red blood cells increased irregularly through the incubation period and into the acute phases of anaplasmosis. The mobilities decreased almost to normal levels as the percentage of infected erythrocytes began to increase. This change in electrophoretic mobility resulted from altered surfaces on red blood cells which in turn increased the net surface charge. These workers indicated that an increase in the electrophoretic mobility of circulating erythrocytes was involved in the pathogenesis of anaplasmosis. It was clear only that the increase in electrophoretic mobility reflected a more negative net charge during the disease. The significance of these alterations has not been determined. It is possible that these changes are important in removal of parasitized or damaged erythrocytes from the peripheral blood and in the host defense against Anaplasma infection. These workers could not offer evidence on the factors responsible for the altered surface properties until a new study was conducted by Dommert and Dimopoullos in 1967 to determine the sialic acid concentration in erythrocytes infected with A. marginale. They concluded from this study that the sialic acids are the primary factors responsible for the surface charge of erythrocytes.

Sialic Acids. As previously stated, Dommert and Dimopoullos (1967) conducted studies on the electrophoretic mobilities of erythrocytes infected with A. marginale and measured the sialic acid concentrations in erythrocytic stromata. A variable increase in electrophoretic mobilities was observed as the disease progressed. Concentrations of sialic acids in stromata increased, paralleling the

rise in electrophoretic mobilities early in the infection. Just before the Anaplasma count reached peak values, the sialic acids began to decrease in concentration whereas the electrophoretic mobilities continued to increase.

Antigens

It has been concluded that the Anaplasma organism contains at least one corpuscular and one soluble antigen, and that these antigens are serologically distinct (Ristic and Mann, 1963).

Erythrocytic Soluble Antigens. Ristic (1962), using disintegration by sonic oscillation and differential centrifugation of Anaplasma-infected erythrocytes, obtained two distinct antigens; a sedimentable one was termed S antigen, and a nonsedimentable one was termed the NS antigen.

The S antigen is a purified preparation of the marginal and initial Anaplasma bodies. The S antigen has been used by Ristic (1962) in a capillary tube agglutination (CA) test for the detection of a serum antibody developed in Anaplasma-infected animals. The NS antigen is apparently a homogeneous substance found between and around the initial Anaplasma bodies. Depending upon the name of the reagent used to extract the NS antigen from infected erythrocytes, designations such as PS or HCl antigen are used (Ristic, Mann, and Kodras, 1963).

Erythrocytic Corpuscular Antigen. The complement-fixation test has been established as a useful tool for diagnosing bovine anaplasmosis (Heck, Franklin, and Huff, 1962). Various methods have been employed in the preparation of antigens used in this test (Weinman and Ristic, 1968). According to Franklin, Heck and Huff (1962a) three factors are

most important in successful antigen production: (1) a large volume inoculum of infected erythrocytes; (2) a highly infected inoculum - 70 percent or higher infected erythrocytes; and (3) maintenance of an environmental temperature of 72 to 75°F for the inoculated animal.

Rogers, Hidalgo, and Dimopoullos (1964) developed a method for preparation of a desirable Anaplasma CF antigen which involved disintegration of infected erythrocytes by sonic vibration and separation of the antigen by differential centrifugation.

The work of Rogers and Dimopoullos (1965) showed that their CF antigen was a lipoprotein. By electron microscopic examination of the CF antigen, they showed that it was not composed of marginal Anaplasma bodies but rather of amorphous membranous material which probably constituted the outer coating of the marginal body.

Ristic and Kreier (1963) reported that, unlike the CF antigen, the antigen used in the capillary tube agglutination (CA) test appeared to consist of round or oval bodies, 0.2 to 0.4 u in diameter; they resembled the initial Anaplasma bodies when viewed under the electron microscope.

Amerault and Roby (1964) reported that using the agar double diffusion (AD) technique, a soluble Anaplasma exo-antigen was found free in serum and in red blood cells (RBC) of cattle with acute anaplasmosis. The exo-antigen was detectable in serum from cattle with acute anaplasmosis within 24 to 48 hours after the peak of parasitemia. The exo-antigen was only present in serum for brief periods near the peak parasitemia. This antigen, purified and freed of hemoglobin by diethylaminoethyl (DEAE) cellulose chromatography, remained serologically active after lyophilization. In zone and immunoelectrophoresis

the antigen migrated toward the anode to a position usually characteristic of either lipoproteins or glycoproteins.

Weinman and Ristic (1968) stated that, according to Theiler (1911), recovery from A. centrale infection resulted in an increased resistance, but not an absolute immunity, to A. marginale. Based upon Theiler's work, A. centrale infection has been used as a premunization method against A. marginale for many years but with variable success. Among other problems, the premunization procedure was responsible in some cases for spreading bovine hemotropic infections other than anaplasmosis (Weinman and Ristic, 1968).

Kuttler (1966) studied the pathogenesis and comparative virulence of A. marginale and A. centrale. A. centrale produced a milder clinical response than did A. marginale, and the infections were accompanied by a serologic response to A. marginale complement-fixation antigen. A. centrale infections resulted in lower serum titers than did those produced by A. marginale infections. Schindler, et al., (1966, cited by Weinman and Ristic, 1968) concluded that A. marginale and A. centrale each possessed at least one unique antigen not shared by the other parasite. These conclusions were reached from a study using the complement-fixation (CF), the capillary tube agglutination (CA) and the Coons indirect fluorescent antibody (CIFA) tests.

Kreier and Ristic (1963c) demonstrated by means of fluorescein-labeled antibody and cross-immunity studies with premune cattle that the round marginal body class of parasite which occurs in erythrocytes of cattle infected with the Oregon strain of Anaplasma is antigenically identical to the parasites which occur in the erythrocytes of cattle infected with the Florida strain. The class of parasites characterized

by a head, body and long tail (Paranaplasma caudata) which occurs in the erythrocytes of cattle infected with the Oregon strain was demonstrated to be antigenically and immunoserologically distinct.

Immunity

Roby and Gates (1962) have stated that infected carrier animals are recognized as having considerable resistance to reinfection. However, such animals can serve as the source for further dissemination of the disease. When carrier animals are free of Anaplasma infection by treatment, they are again susceptible. The immunological problem in anaplasmosis is the absence of immunity after recovery from their carrier state.

According to Weinman and Ristic (1968) there are four states of resistance or immunity in infection with A. marginale: (1) innate (species) resistance which is a state of nonsusceptibility to infection characteristic of some species; (2) natural resistance in which some individuals of susceptible species such as deer, and calves do not develop clinical signs of the disease; (3) idiopathic resistance which is the resistance of unknown origin possessed by some cattle. It is observed in animals one to two years old after inoculation with certain amount of Anaplasma-infected blood known to produce an acute infection in cattle of similar age; and (4) acquired immunity which, in the case of anaplasmosis, is the resulting immune state of an animal exposed to Anaplasma antigens.

Premunition. Premunition is an immunity which depends on the persistence of a latent infection in animals which survive an initial Anaplasma infection. The immune response developed in premunition is characterized by the production of serologically detectable antibodies which can be demonstrated by the CF, the CA, or the gel precipitation (GP) tests (Weinman and Ristic, 1968).

Active Immunity. Attempts have been made to stimulate an immune response in susceptible cattle by inoculating them with various types of killed Anaplasma preparations. Attempts to induce clinically useful immune responses in susceptible mature cattle have been unsuccessful using whole blood, washed erythrocytes, the complement-fixing (CF) Anaplasma antigen, the Anaplasma antigen used in the capillary tube agglutination (CA) test, the soluble erythrocytic antigen known as "protamine sulfate"(PS) antigen, and the free serum antigen known as "exo-antigen" (Weinman and Ristic, 1968).

Brock, Kliewer and Pearson (1965a) developed an inactivated vaccine prepared from Anaplasma-infected bovine whole blood which increases the resistance of Anaplasma-infected cattle to clinical anaplasmosis and prevents death from the infection. The vaccine[1] is now commercially available (Brock, Kliewer, and Pearson, 1965b).

The antigen used in the vaccine was prepared from Anaplasma-infected bovine blood and most of the plasma and cellular material was removed. The remaining antigenic material was freeze-dried. The best adjuvant used as a vehicle for the antigen appeared to be an oil and water emulsion. The vaccine should be administered in two doses

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[1] Anaplaz, Fort Dodge Laboratories, Fort Dodge, Iowa

separated by an interval of about six weeks. Since it is an inactivated vaccine (contains killed organisms which will not produce anaplasmosis), revaccination is needed in subsequent years. Vaccinated cattle become carriers when challenged with either 0.1 or 0.01 ml of anaplasmosis carrier blood (Brock, Kliewer, and Pearson, 1965a).

Ristic, Sibinovic, and Welter (1968) reported the development of an attenuated A. marginale vaccine by selecting an avirulent variant of the organism in an atypical host. The selection of the avirulent A. marginale variant was made by serial passages of irradiated organisms in nonbovine ruminants including deer and sheep.

Kuttler, Zaraza and Roberts (1968) conducted one study using Anaplaz[2] vaccine and the attenuated A. marginale vaccine in Colombia, South America. They concluded that no significant evidence of protection was produced by either the Anaplaz[3] vaccine or the attenuated A. marginale vaccine in the 3-month-old calves and adult cattle used in the experiment.

Passive Immunity. According to Weinman and Ristic (1968) convalescent bovine Anaplasma sera have been used in an effort to demonstrate the protective effect of antibody against Anaplasma, but the serum antibody per se is not effective in controlling anaplasmosis. This conclusion was made based on one experiment in which sera from cattle convalescent from anaplasmosis were mixed with Anaplasma-infected blood and injected into susceptible cattle. It was observed that the mixture retarded the appearance of Anaplasma bodies in the inoculated animals, but did not affect the appearance of anemia and clinical manifestations

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[2] Ibid., p. 30

[3] Ibid., p. 30

of the disease.

Pathogenesis

Anaplasmosis, which is an infectious disease, produces rapidly progressive anemia in adult cattle (Brock, et al., 1959). The resulting syndrome of the infection may be divided into a prepatent period, a period of increasing anemia, and convalescence (Ristic, 1970). The prepatent period ranges from three to six weeks (Ristic, 1960), and the location of A. marginale during this period is unknown (Pearson, 1965).

Patent disease is manifested by Anaplasma bodies within the circulating red blood cells. It has not been established by what manner erythrocytes become parasitized, but invasion of mature red blood cells by initial bodies has been postulated (Ristic and Watrach, 1963). Maximal anemia occurs 1 to 3 days after peak parasitemia (Brock, et al., 1959). Removal of infected host cells is thought to result from phagocytosis by the reticuloendothelial system, especially the spleen (Kreier, Ristic, and Schroeder, 1964). The fate of the Anaplasma body after phagocytosis is unknown but it has been suggested that the marginal body breaks down into initial bodies which will perpetuate the infection in other erythrocytes (Ristic, 1960).

The increase in the number of infected erythrocytes is rapid, approximately doubling daily for several days. Increasing anemia persists for 5 to 10 days. The degree of the parasitemia depends upon the animal's resistance.

Accelerated hematopoiesis induced by the anemia is indicated by reticulocytosis, macrocytemia, and granulopoiesis. The degree of this hematopoietic response is usually maximal between the second and third

weeks of patent disease. Convalescence usually lasts 1 to 2 months (Jones and Brock, 1966).

Clinical Findings. Animals of all ages are susceptible, but the severity of the resulting syndrome is directly related to age. In animals less than one year old, anaplasmosis is usually sub-clinical; in yearlings and two-year-olds, it is of moderate intensity; and is severe, frequently fatal, in older cattle (Jones and Norman, 1962). The reason for the age-related disease intensity is unknown, but it is possibly related to the efficiency of the reticuloendothelial system (Jones and Brock, 1966). A syndrome of moderate intensity has been observed in cattle native to enzootic areas (Jones and Norman, 1962).

Pyrexia is the first sign observed. The fever varies in degree, often exceeds 105°F (40.6°C) and usually persists throughout the period of increasing parasitemia (Jones and Norman, 1962). Subnormal temperatures have been reported prior to death (Jones and Brock, 1966).

Subsequent clinical findings in cattle with anaplasmosis result from erythrocyte loss. There is pallor of the skin, nose, udder, mucous membranes of the eye, and vulva in the female. The anemia causes weakness and depression and requires physiologic adjustment of the respiratory and circulatory systems. Such physiological adjustment includes altered heart rate and incrased pulmonary ventilation. Although tachycardia occurs in animals which have lost as few as 25% of their erythrocytes, a marked increase in cardiac rate does not occur until more than 50% of red blood cells are destroyed. In such cases, cardiac auscultation reveals systolic murmurs and more intense heart sounds. Tachypnea similarly occurs, is maximal when anemia is most severe, and is most apparent when the animals are forced to move (Jones and Norman, 1962).

Other clinical signs include gastrointestinal atony, inappetence, lowered production, weakness and recumbency. In advanced cases, muscle tremors, weight loss, and dehydration are common. Some animals become restless and excited, perhaps due to cerebral anoxia. Dehydration suppresses gut motility and this, along with inappetence, is probably responsible for the weight loss and constipation. The feces are frequently bile-stained, probably reflecting elevated fecal urobilinogen values. Animals infected in advanced pregnancy frequently abort. Temporary infertility has been observed in bulls (Jones and Norman, 1962).

Icterus is a late sign of the disease and is most frequently observed during early convalescence (Brock, et al., 1959). Myocardial anoxia occurs, and obesity and stress, such as that resulting from struggling against restraint, predispose the heart to failure (Jones and Norman, 1962). The onset of cardiac failure is indicated by weakness, tremors, an accelerated irregular heart-beat, a weak pulse, pulmonary rales, dyspnea and tachypnea, sometimes a subnormal temperature, and jugular distension during transfusion. Death often results from cardiac failure. The mortality varies from few if any in young to 50% to 60% or more in adult and aged animals (Jones and Brock, 1966).

Clinical convalescence is manifested by return of the appetite and temperature to normal and by resumption of normal intake of water. Some convalescent animals have depraved appetites and eat soil (Jones and Norman, 1962). Recognition of early convalescence is important, because treatment at this time can be extremely hazardous (Brock, et al., 1959).

Necropsy Findings. Gross pathological changes are typical of an acute anemia. The prominent lesions include icteric mucosae, enlarged spleen and distended gall bladder.

Histologically there is evidence of hepatic, renal and myocardial degeneration, hemosiderosis and erythrophagocytosis. The bone marrow is usually hyperplastic, but in chronic cases there may be evidence of depletion (Ristic, 1970).

Diagnosis. The diagnosis of anaplasmosis is based upon many factors and the following are of major importance: geographic location; season; age of the animals; and clinical, laboratory and necropsy findings (Jones and Brock, 1966). Knowledge of the geographic location of anaplasmosis is desirable when susceptible cattle are introduced into an enzootic area (Jones and Brock, 1966). The seasonal incidence is related to insect vector populations (Jones and Brock, 1966), and is most frequently observed during summer and fall (Baker, Osebold, and Christensen, 1961). Mechanical transmission of anaplasmosis produced by surgical instruments or hypodermic needles may occur at any time. Clinical anaplasmosis is usually observed in cattle one to two years old or older, and mortality is more likely in mature and aged animals (Jones and Brock, 1966). The most significant clinical findings are fever, pallor, weakness, constipation, normal urine and watery blood (Jones and Norman, 1962). In contrast, hemoglobinuria is common in certain other diseases.

The differentiation of clinical anaplasmosis from other causes of acute anemia is made by microscopic examination of the stained blood smear. Other causes of acute anemia are leptospirosis, bacillary hemoglobinuria, babesiosis and post-parturient hemoglobinuria (Jones and Brock, 1966). Conditions such as clostridial infections, anthrax, lightning stroke and acute poisoning must be excluded when the owner complains of fatalities (Jones and Brock, 1966).

Staining. Microscopic examination of the blood permits confirmation

of the diagnosis and is essential for adequate prognosis (Brock, et al., 1959). Blood smears may be stained by several methods of which Giemsa staining is the most frequently used (Ristic, 1970). According to Gainer (1961) the detection of A. marginale by the conventional Wright's or Giemsa's staining procedure presents doubts even for the experienced worker, especially where the percentage of infected cells is low and where dye deposits may be confused with the parasite.

Smears should be examined both for erythrocytes and for evidence of bone marrow response. Detection of parasitemia is of value only during the acute disease since it is not found in the carrier animal (Jones and Brock, 1966). Rogers and Wallace (1966) described a one-step toluidine blue staining technique useful for rapid detection of Anaplasma in erythrocytes in the field or laboratory. Schalm (1967) describes the use of new methylene blue stain in physiologic saline solution on dry unfixed blood films for the detection of A. marginale in erythrocytes. Romanowsky stains are frequently employed for identification of A. marginale in the blood and other tissues of infected cattle (Ristic, White and Sanders, 1957). Gainer (1961) used the fluorescent dye, acridine orange (AO), for the demonstration of A. marginale in formalin-fixed erythrocytes. This technique has, according to Weinman and Ristic (1968), the disadvantage of staining nucleic acids, and both immature erythrocytes and A. marginale contain them. One source of confusion which often exists is Howell-Jolly bodies (Gainer, 1961). These structures have been defined by Schalm (1967) as small, spherical, eccentric nuclear remnants in young erythrocytes. In Wright's-stained smears they appear, according to Coles (1968), as single and sometimes double spherical bluish bodies located anywhere within a red blood cell but are not confined to the

periphery as Anaplasma bodies. Gainer (1961) has stated that the size of Howell-Jolly bodies, which is two or more times larger than A. marginale, and their central or eccentric location in the erythrocyte rather than the peripheral position of the Anaplasma body, should differentiate the two structures. Coles (1968) states that Howell-Jolly bodies are not usually surrounded by a halo as are Anaplasma bodies. Anaplasma bodies are often confused with artifacts. Both the parasites and Howell-Jolly bodies remain in focus as long as the erythrocyte edge is in focus, whereas artifacts usually turn light in the center when slightly out of focus.

Ristic, White and Sanders (1957) describe the use of a fluorescent antibody (FA) technique as a means of detecting A. marginale in the blood of cattle suspected of harboring the agent.

Evidence of an immune response is also a good diagnostic tool and can be detected by the complement-fixation (CF) and capillary-agglutination (CA) tests (Ristic, 1962). These two serologic tests are now used rather generally to detect carrier animals (Safford, 1966). Complement-fixing Anaplasma antibodies are detectable in infected cattle beginning at 10 to 25 days after experimental inoculation (Ristic and Mann, 1963). The CF test has been used to identify carriers of anaplasmosis accurately (Jones and Brock, 1966). Reliability comparable to that of the CA test has been reported by Pilchard and Ristic (1963). Agreement between the CA and CF tests was reported to be 91% (Welter, 1966), but transient negative CF tests may result from tetracycline therapy (Franklin, Heck, and Huff, 1962b). Some animals treated with tetracyclines to eliminate the carrier state have been observed to remain CF positive for several months despite successful therapy

(Riemenschneider, 1964). Some serum samples contain anticomplementary substances which interfere with the CF reaction and necessitate retesting. Hemolysis may also make this test difficult to interpret (Pilchard and Ristic, 1963).

The CA test, developed by Ristic (1962), has been reported to be positive beginning 30 days following inoculation of cattle with A. marginale (Pilchard and Ristic, 1963). The earliest reactions are observed before the peak of acute infection; in the majority of cases the reactions are evident when Anaplasma bodies are present in 1 to 3% of the erythrocytes (Ristic, 1962). After successful elimination of the carrier state, the CA test may become negative more rapidly than the CF test (Riemenschneider, 1964). According to Amerault and Roby (1968) the CF test is tedious to perform and considerable technical knowledge and skill are required. The CA test, although easily performed, requires serum inactivation and incubation for 24 hours before final readings can be made.

Amerault and Roby (1968) described a method for preparing an agglutinating antigen to detect carrier cattle of anaplasmosis. They claim that the reaction obtained with this antigen is very rapid requiring no more than five minutes and a minimum of equipment. These advantages make this test adaptable to field conditions. The antigen is composed of A. marginale organisms liberated from the erythrocytes by passage through the French pressure cell using 1,200 to 1,500 lbs. of pressure. The Anaplasma parasites are washed in saline solution, treated with antibiotics, and the parasites free of hemoglobin are stained with fast green dye for use in a card agglutination test.

Prognosis. Prognosis is adversely influenced by maturity and

advanced age and by pregnancy, lactation, and stress during the period of severe anemia. Clinical icterus usually indicates that severe anemia has been present for longer than 24 hours, that convalescence has commenced, and that cardiac failure may easily result from additional stress. Drinking and return of the appetite are favorable prognostic signs, as is hematological evidence of bone marrow activity (Jones and Brock, 1966).

Treatment. The infective agent and the resulting anemia are factors which require consideration when an animal with clinical anaplasmosis is treated (Jones and Brock, 1966). The successful treatment of cattle acutely affected with virulent strains of A. marginale depends on an early diagnosis and early treatment. Many acute cases which have been treated only once, have ended fatally. Some animals should be treated two or three times at 12 to 24 hour intervals (Foote, 1968).

To date, only the tetracyclines (chlortetracycline, tetracycline, and oxytetracycline) have been effective in controlling the rate of multiplication of Anaplasma and altering the course of the disease (Ristic, 1970). Parenteral doses of 3 to 5 mg/lb of body weight are used (Franklin, Heck, and Huff, 1962b). These drugs are not helpful when used during or later than the maximal anemia (Miller, 1953).

Treatment of cattle when their hemoglobin (Hb) values are greater than 4.0 gm/100 ml provides 100% survival, but when hemoglobin values are below 4.0 gm, only 85% survive (Miller, 1956).

Klaus, Jones, and Kliewer (1967) reported that five splenectomized steers which were carriers of A. marginale were given 3 doses of 14.3 mg of estradiol cypionate per 100 kg of body weight. This resulted in rapid disappearance of parasitemia in four of the steers, a situation

that lasted 6 to 7 days. The mechanism of this phenomenon is unknown; it may reflect a temporary hyperphagocytic state of the extra-splenic reticuloendothelial system (RES) or a direct parasiticidal action on the Anaplasma organism.

Brown, Wilde, and Berger (1968) reported suppression of A. marginale multiplication, control of developing anemia, and prevention of death using single intravenous injections of 5 mg/kg of dithiosemicarbazone in experimentally infected splenectomized calves and intact, susceptible steers. Roby, Amerault, and Spindler (1968) reported a marked inhibiting activity of dithiosemicarbazone against A. marginale in adult cattle and splenectomized calves, using a single intravenous dose at a rate of 5 mg per kg of body weight. Kuttler and Zaraza (1970) reported that dithiosemicarbazone, when administered early in the course of infection as a single intravenous injection at the rate of 5 mg/kg, was effective in preventing death loss and in reducing the severity of infection. Kuttler and Adams (1970) compared the efficacy of oxytetracycline and dithiosemicarbazone in eliminating A. marginale infection in splenectomized calves. Oxytetracycline was given at the rate of 11 mg/kg intravenously for 5 and 10 days consecutively. Dithiosemicarbazone was given at the rate of 5 mg/kg intravenously for 5 and 10 consecutive days. They found that dithiosemicarbazone was relatively more effective than oxytetracycline in the treatment of anaplasmosis when administered for 5 consecutive days. Dithiosemicarbazone administered daily for 10 consecutive days, however, resulted in atony of the rumen, tympanitis and death.

Treatment of acutely ill cows by blood transfusion has been used (Franklin, Heck and Huff, 1962b). Slow transfusion of 2 to 4 liters

has been recommended to be repeated once or twice, preferably within 24 hours. This approach should be used only in gentle animals. It is contraindicated when restraint is difficult, when the animal is restless or when its mucous membranes are icteric. Transfusion may cause additional cardiac embarrassment, marked jugular distension, severe tachycardia, and pulmonary congestion. It is also possible that blood transfusion induces recrudescence of the parasitemia (Jones and Brock, 1966). According to Foote (1968), the digestive system should be activated as quickly as possible. The use of parenteral peristaltic stimulants will activate the digestive system and will also help to evacuate the gall bladder. Such drugs should be used cautiously in pregnant cows. The administration of five to ten or more gallons of water via stomach tube with one pound of epsom salts will be of benefit in relieving both dehydration and constipation.

The use of other supportive therapy is of questionable value. Hematinic drugs, for example, probably do not have time to stimulate erythropoiesis to influence the mortality, but they may reduce the time required for convalescence (Brock, et al., 1959).

Control. The control of anaplasmosis requires consideration of reservoirs of infection, the mode of transmission, innate and acquired resistance, and therapy (Jones and Brock, 1966).

The most potent source of infection to other animals is the cow with acute anaplasmosis. Persistence of the organism is largely dependent upon the carrier animals (Jones and Brock, 1966).

According to Howell (1957) ticks act as a source of infection and their ability to carry Anaplasma organisms for years is very important in disease control procedures.

The practice of separating infected from non-infected animals, after identifying infected animals by CF and CA tests, may be useful in the areas where mechanical transmission, such as that by horseflies and mosquitoes, predominates (Ristic, 1970). Cattle with inconclusive and anticomplementary reactions should be retested.

Reactor cattle should be sold for slaughter, isolated or treated with tetracyclines (Jones and Brock, 1966). Calves born of carrier cattle are not initially infected but, due to colostral antibody, are CF positive for up to 50 days (Kuttler, 1962).

In areas where ticks transmit the disease and wild reservoirs exist, the practice of testing and isolation is less effective than in areas where mechanical transmission predominates. In the areas where ticks transmit the disease the infection in cattle appears to be maintained at high levels because of host parasite relationships involving deer, ticks and cattle (Ristic, 1970).

Efforts to control anaplasmosis by vector elimination have had little success (Ristic, 1970). Factors such as terrains, climate, and vegetation contribute to the propagation of large numbers of ticks and wild mammals which serve as hosts for them (Rea, 1965). This worker has also reported that control of insect vectors by spraying has been tried in some areas, but rough terrains prevent a practical application on a wide scale at least in the Northwest part of the United States. Automatic spraying around tanks or watering holes was effective in some areas but not in the Northwest because animals have access to rivers, creeks and wooded areas where vector control is not possible. Tick control is difficult due to rough terrains and the multiple host characteristic of Dermacentor species. According to Safford (1965), the application of

pesticides to kill ticks, even in small areas, is almost impossible due to varieties of terrains. Attempts to use rodenticides to deprive the three-host ticks of their larval and nymphal stages has been found unsatisfactory. Adequate control of biting flies and mosquitoes during the summer is only remotely possible when terrains are rough and grazing lands inaccessible.

Varying degrees of resistance to anaplasmosis occur. In young cattle an innate resistance exists and in older cattle varying degrees of resistance are observed. Premunition is common in cattle which have recovered from anaplasmosis, and is characterized by persistent latent infection and resistance to subsequent challenge (Jones and Brock, 1966).

According to Brock (1965), there are at least 3 possible immunizing procedures against anaplasmosis: (1) a living, fully virulent organism; (2) an attenuated living organism; and (3) a killed Anaplasma organism. Use of the fully virulent, living A. marginale, in combination with antibiotics to control infection, may be used to premunize against anaplasmosis, but it is not a recognized practice in the United States. The inherent dangers and disadvantages restrict its use.

A. centrale, which has not been reported in the United States, is the only organism which will produce an infection with moderate signs and yet provide prolonged resistance to the more severe signs produced by A. marginale. An attenuated organism, should one become available, could be transmitted naturally from animal to animal.

A degree of resistance has been induced by use of a killed Anaplasma antigen, an inactivated vaccine[4] developed by Brock, Kliewer

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[4] Anaplaz, Fort Dodge Laboratories, Fort Dodge, Iowa

and Pearson (1965b). The vaccine must be given initially in two doses because one dose proved not to be effective, according to these workers. Six weeks have been set as the minimum interval between the two doses of vaccine, but intervals as short as 2 weeks, and as long as 19 weeks have also been reported to be effective. The second dose of vaccine causes antibody response significant enough to produce a positive reaction to the CF test for 1 to 10 months. According to Jones and Brock (1966), the development of maximum resistance requires approximately two months after initial vaccination.

Kuttler (1961) has stated that injections of Anaplasma antigen prior to infection with anaplasmosis reduce the clinical symptoms and death losses from the disease. Kuttler (1962) reported that vaccination with killed Anaplasma organisms with adjuvants reduces the severity of clinical anaplasmosis but will not prevent development of the carrier state. Kuttler and Adams (1970) reported that positive reactions to the CF test and the fact that the vaccine does not prevent anaplasmosis infection, have been objections to its use.

Dennis, et al., (1970)reported an isoimmunohemolytic disease characterized by anemia and icterus which they believed to be related to use of Anaplaz[5] vaccine. In some affected herds, 15 to 24% of all newborn calves died from this disease. Most of the calves became anemic and jaundiced within 24 to 48 hours post-partum and died during the first week of life. Lesions observed in these calves included anemia, icterus, and marked splenomegaly. A few less severely affected calves survived the acute episode and returned to normal health in 2 to 3 weeks. Some

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[5] Ibid., p. 43

calves died within 24 hours of birth of a peracute form of the disease in which the principal clinical sign was dyspnea and the lesions were pulmonary edema and splenomegaly.

Dams of affected calves were all inoculated with Anaplaz[6] vaccine. The sera from dams of affected calves contained antibodies which agglutinated and lysed the sires' erythrocytes.

Further work needs to be done to determine the type of antigens involved in the pathogenesis of this disease and type of antibodies contained in sera of vaccinated cattle.

The carrier state of anaplasmosis has been eliminated by daily intramuscular injection of a tetracycline, 5mg/lb of body weight, for 10 days or a similar dose administered orally for 60 days. The acute disease has been prevented giving 0.1 mg/lb of body weight of the same drug as a feed supplement (Brock, 1959), or using intramuscular injection of 1 to 2 mg/lb of body weight every 3 to 4 weeks (Miller, 1962).

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[6] Ibid., p. 43

CHAPTER III

CONCLUSIONS

Anaplasma marginale has been studied extensively and a great amount of data are available concerning the organism and the disease it produces. However, many questions remain concerning the basic structure, histochemical nature and invasive mechanism of the organism. Likewise, controversy surrounds the mechanism of reproduction; some evidence supports budding while other data suggest that binary fission is the means of replication. In the absence of such information its proper biological classification is still controversial. Its life cycle and activity within arthropod vectors, its location in cattle during the prepatent period and a reason for the relationship between the age of the affected animal and the intensity of the disease are in need of investigation.

Some important aspects are well known in anaplasmosis: the initial Anaplasma body is the initiator of the infectious cycle; the organism can be stored for research purposes by freezing in liquid nitrogen; its infectivity can be reduced by irradiation. Nevertheless there are fundamental aspects of the biology in this parasite which must be elucidated before it can be controlled.

Only ticks and horseflies are considered important vectors of the disease. There is evidence that viable Anaplasma organisms can pass through the various stages of tick development and by transovarian

passage. The true reservoir of the disease is infected cattle although certain wild ruminants apparently can also serve as a source of infection.

Anaplasma centrale has been used in many countries for premunization against A. marginale infection. This intentional establishment of carrier animals by premunization has not been accepted generally as a good practice because of the danger of unintentionally transmitting other blood parasites and viruses. Carrier animals have a considerable resistance to reinfection but when they are freed of infection by treatment they are again susceptible and may become carriers. Duration of immunity in cattle after recovery from acute anaplasmosis has not been determined.

Elimination of the carrier state by tetracycline therapy is readily accomplished. In countries like Colombia, however, this approach to control offers no solution at this time because of cost of drugs and difficulties in control of animate vectors which may carry Anaplasma organisms to the areas where tetracyclines have been used.

The only presently available vaccine does not protect against Anaplasma infection but does prevent clinical manifestations and death losses. From this point of view any vaccinated animal is a potential carrier and this is a serious deficiency of the vaccine.

The value of using vaccines against anaplasmosis has not been determined adequately for Colombia. Since it has been concluded that protection produced by neither the commercially available killed vaccine nor an attenuated one was significant in animals used in studies conducted in Colombia, there is evidence that further studies need to be done to determine the value of these vaccines in Colombia. Surveys should continue around the world to evaluate the present vaccines and

also research should continue in order to develop new vaccines. No vaccines are available at the present time in Colombia.

Inoculation of virulent A. marginale organisms into young calves could be a practical and economical method to reduce losses from this disease. This practice is not recommended from the scientific point of view because it means deliberate spreading of the disease producing many more carrier animals than would occur naturally. However, this may be the price to pay in developing countries to reduce the severe economic losses from this disease. Exposure of the young calves to anaplasmosis when they are resistant to severe clinical manifestations of the disease will protect them from acute disease later on.

Control of the disease is very difficult because of the presence of a number of animate vectors for which there are no practical means of control. In tropical zones where vectors are active all year due to favorable climatic conditions, control of anaplasmosis is more difficult and the disease can be transmitted anytime. In these areas any bovine animal is a possible carrier which makes control of the disease more difficult than in zones where animate vectors are not active all year.

Effective treatment for anaplasmosis awaits further research. Only the tetracyclines are effective in acute anaplasmosis (if used in the developmental stage of the disease), and they are also effective in eliminating the carrier state at low levels. The group of chemicals known as dithiosemicarbazone has also been reported to be effective against A. marginale. Efforts should be continued to develop this and other drugs which can be used to control effectively and economically the acute and the carrier state of the disease.

The CF and the CA tests used to detect infected animals are not

employed in developing countries like Colombia because they are not available at the present time. However, surveys should be conducted using these tests to determine the incidence of anaplasmosis in Colombia and map out the enzootic and non-enzootic areas. From this information the prevalence of anaplasmosis, its morbidity, mortality and economic impact can be accurately evaluated, and the information will be useful in the future, especially if new methods to control anaplasmosis can be developed.

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VITA

Mario Perez

Candidate for the Degree of

Master of Science

Report: A REVIEW OF ANAPLASMOSIS

Major Field: Veterinary Parasitology

Biographical:

Personal Data: Born in Entrerrios, Colombia, September 16, 1943, the son of Antonio Perez and Edelmira Palacio.

Education: Graduated from Liceo Antioqueno High School in 1961; received the Doctor in Veterinary Medicine in July, 1967 from Universidad Nacional, Bogota, Colombia; completed requirements for Master of Science in May, 1972.

Professional Experience: Instructor, Universidad de Antioquia, 1967 to 1968; Assistant Professor, Universidad de Antioquia from April 1968 to present.

Honorary and Professional Societies: Phi Zeta, American Veterinary Medical Association.