

STUDIES ON BOVINE BESNOITIOSIS

WITH SPECIAL REFERENCE TO

THE AETIOLOGY

J. W. POLS

ONDERSTEPOORT LABORATORY

Submitted in partial fulfilment of the  
requirements for the Degree of Doctor  
of Veterinary Science, in the Faculty  
of Veterinary Science.

UNIVERSITY OF PRETORIA. DECEMBER, 1959

## C O N T E N T S

I.	INTRODUCTION . . . . .	3
II.	DEFINITION . . . . .	4
III.	SYNONYMS . . . . .	4
IV.	HISTORY. . . . .	5
V.	DISTRIBUTION . . . . .	7
VI.	AETIOLOGY. . . . .	9
	{a} Morphology . . . . .	9
	{b} Multiplication . . . . .	.16
	{c} Habitat. . . . .	.17
	{d} Life Cycle . . . . .	.22
	{e} Cultivation. . . . .	.22
	{f} Resistance to Physical and Chemical Agents. . . . .	.22
	{g} Biological Characteristics . . . . .	.25
	{h} Maintenance of the <u>Besnoitia</u> <u>besnoiti</u> Strain. . . . .	.25
	{i} Classification . . . . .	.26
VII.	TRANSMISSION . . . . .	.51
	{a} Natural transmission . . . . .	.51
	{b} Artificial transmission. . . . .	.52
VIII.	EPIZOOTOLOGY . . . . .	.58
IX.	PATHOGENICITY. . . . .	.59
X.	PATHOGENESIS . . . . .	.63
XI.	SYMPTOMATOLOGY . . . . .	.65
	{a} Besnoitiosis in ruminants . . . . .	
	{b} Besnoitiosis in rabbits . . . . .	
XII.	DIAGNOSIS. . . . .	.74
XIII.	TREATMENT. . . . .	.75
	(a) Treatment during period of re- action. . . . .	.75
	(b) Treatment during the incubation period and period of reaction . . . . .	.77
XIV.	PROPHYLAXIS . . . . .	.80
XV.	IMMUNITY . . . . .	.81
XVI.	SUMMARY AND CONCLUSIONS. . . . .	.82
XVII.	ACKNOWLEDGMENTS. . . . .	.84
XVIII.	REFERENCES . . . . .	.84
XIX.	ILLUSTRATIONS. . . . .	94
XX.	APPENDIX . . . . .	106

## INTRODUCTION.

Bovine besnoitiosis was recognised as a distinct disease in South-western Europe about 75 years ago. The discovery of the causative protozoon in the skin and subcutaneous tissues 38 years later in France opened several new fields of investigation. These included ascertaining the distribution of the disease, identifying the protozoan parasite, establishing the pathogenicity, determining the seriousness of besnoitiosis as an animal health and economic problem, looking for economically feasible methods of controlling the disease, attempting to determine the natural mode of transmission, and searching for chemotherapeutic agents for either curing or preventing the disease. Consideration of the results of the investigations, described below, makes it apparent that although advances have been made in the studies during the last five decades, the failure of establishing the natural mode of transmission, and the absence of a specific remedy leaves bovine besnoitiosis as an important veterinary and economic problem.

In presenting the results of the investigations an attempt has been made not only to give a detailed account of the aetiology but to include observations made on the pathogenicity, symptomatology, chemotherapy and immunology as they reveal the biological properties of the infectious agent. For the sake of completeness a brief review on the epizootology, diagnosis and prophylaxis will also be given. In doing so it will become clear what has been achieved, and which avenues to future progress have to be followed.

Advances made in the studies on the aetiology during recent years have made it possible to dissociate the causal agent of bovine besnoitiosis from the eimerian parasites, the association of which, in the past, had led to a hopeless classificatory and nomenclatorial confusion. It has at last become possible to give a clear definition of bovine besnoitiosis, and the two allied diseases occurring in equine and murine hosts.

/2.....

Since the presence of bovine besnoitiosis was first recognised in the Rustenburg district nearly twenty years ago, an alarming spread to the adjoining districts has occurred in the Northern Transvaal, which constitutes one of the main ranching areas of the Union of South Africa. The economic implications of bovine besnoitiosis to stock owners are considerable. Although the direct losses do not exceed ten per cent, the indirect losses as result of poor productivity due to the very long convalescent period, the temporary or permanent sterility of affected bulls, and the heavily parasitized hides rendered useless for the leather industry are extremely high.

#### DEFINITION

Bovine besnoitiosis is an acute, subacute or mild protean disease caused by Besnoitia besnoiti (Maretel, 1912). It is characterized by pyrexia, anorexia, malaise, swelling of the palpable lymphatic glands, scleritis, lachrymation, anasarca, sclerodermatitis, alopecia, rhinitis, pharyngolaryngitis, tracheitis and orchitis. Cattle that survive either an acute or subacute infection, retain a chronic sclerodermatitis, rhinitis, pharyngolaryngitis, tracheitis and scleritis accompanied by a permanent or temporary sterility in bulls, and a premunity which persists for life in both sexes.

#### SYNONYMS

Bovine cutaneous globidiosis, Bovine cutaneous sarcosporidiosis, Elephant skin disease of cattle; Huid-sarkosporidiose van beeste, Huidglobidiose van beeste, Besnoitiose van beeste, Olifantsvelsiekte, Dikvelsiekte (Afrikaans); Huidglobidiose van runderen, Besnoitiose van runderen (Netherlands); Haut Sarcosporidiose der Rinder, Haut Globidiose der Rinder, Besnoitiose der Rinder (German); Anasarque du boeuf, Elephantiasis du boeuf, Sarcosporidiose cutanée chez les bovins, Globidiose cutanée du boeuf, Besnoitiose du boeuf (French); Anasarca bovina, Sarcosporidiose cutanea bovina, Globidiose bovina per Globidium besnoiti, Besnoitiose bovina per Besnoitia besnoiti (Portuguese); Dillag (Arabic).

## HISTORY

The history of bovine, equine and murine besnoitiosis is interesting. Perusal of the literature shows that observations conducted on domestic animals in Europe and Africa were accompanied by a remarkable confusion about the identity of the causal agents, the nomenclatorial history of which will be discussed below (Vide infra - Aetiology, Classification).

During the last five decades the disease in cattle was known as either bovine cutaneous sarcosporidiosis (Besnoit and Robin, 1912, 1913 a, b, 1914; Cuille, Chelle and Berlureau, 1936) or bovine (cutaneous) globidiosis (Cuillé and Chelle, 1937; Hofmeyer, 1945; Leitaø, 1949; Pols 1954 a, b; Henning, 1956), and that in horses as equine (cutaneous) globidiosis (Bennet, 1933; Schulz and Thorburn, 1955; Henning, 1956). Recently, however, it became apparent that the correct generic names are bovine and equine besnoitiosis.

A disease referred to as "l' elephantiasis et de l'anasarque du boeuf" was described by Cadéac (1884) in cattle from Southern France. Subsequent investigations by Besnoit and Robin (1912) revealed the presence of an extremely large number of thick-walled spherical cysts, harbouring numerous spores, in the skin and sub-cutaneous tissues of affected cattle from the Pyrenees. They tentatively referred to the causal agent as a Sarcocystis sp. Marotel (1912) pointed out that nothing similar had been found previously in cattle, and that the names of the investigators deserve to be connected with this wonderful discovery. He, therefore, proposed the generic name Besnoitia, and used the combination Besnoitia besnoiti for the causal agent.

Franco and Borges (1916) gave a detailed account of the disease and stated that since 1885, it has been encountered frequently in slaughter cattle at the abattoir in Lisbon, particularly in animals derived from the province Alentejo in Portugal. In their account they reported that not only was the skin affected but that the aponeuroses revealed a massive infection of cysts giving

the impression that these tissues had been sprinkled heavily with sand. Infected carcasses were declared as unfit for human consumption. Since the disease was encountered in the Sudan (Bennet, 1928), and in several other African states listed in the subjoined Table I. (Vide infra - Distribution).

The first record of equine besnoitiosis is that of Henry and Masson (1922) who encountered the infection in a horse in the vicinity of Rheims in Northern France. Several years later Going (1927) and Bennett (1927) encountered this disease in horses from the Sudan. It was later established that the horses originated from the Nuba Mountains situated in the southern part of the province Kordofan. The economic importance of this infection was brought to light by a series of official enquiries carried out by Capt. H.B. Williams in whose opinion the horse-owning tribes were well acquainted with this disease. He reported that the Hawasma Arabs of Rashad Merkez not many years ago possessed 600 to 800 horses, of which less than fifty remained at the time of enquiry. This loss was attributed to a disease which the Arabs named "Dillag", apparently equine besnoitiosis.

Although Babudieri (1932) and Enigk (1934) stressed that the cutaneous parasites of cattle and horses are not related to the intestinal parasites (Globidium spp.) of these animals many investigators persisted in referring to these diseases caused by the former organisms as cutaneous globidiosis.

The turning point in the investigations on besnoitiosis was the discovery of Jellison (1953), who found pearly white cysts, harbouring numerous spores, in the mediastinum of a white-footed mouse (Peromyscus maniculatus artemisae (Wagner)). However, it was left to Frenkel (1953 a,) to show that this parasite, which multiplies by binary fission, is not only related to Besnoitia besnoiti but also to Toxoplasma gondii. These studies were followed by those of Pils (1954 a, b) who found that B. besnoiti also reproduces by binary fission, and that it is morphologically indistinguishable from T. gondii.

## DISTRIBUTION.

The distribution of bovine, equine and murine besnoitiosis together with the names of investigators responsible for determining their presence in different countries are listed in Table I.

From the description given by European investigators it becomes apparent that bovine besnoitiosis occurs enzootically in France and Portugal. Leita<sup>o</sup> (1949) reported that cases of this infection were frequent in cattle imported into Portugal from Angola. Whether or not these animals served as additional reservoirs of B. besnoiti for the infection of indigenous Portuguese cattle could not be determined.

Since the recognition of bovine besnoitiosis in South Africa in 1941 (Hofmeyer, 1945) surveys by Thomas et al (1943) revealed that this disease is widely distributed in the Rustenburg district. Farmers in this area stated that they had seen this disease for the last decade but that they had mistaken it for bovine mange. Subsequent investigations by veterinarians, listed in the appended Table I, have disclosed that the disease is gradually spreading, and at present it is widely distributed in the North-Western, Northern and North-Eastern bushveld area of Transvaal. It has also been established that the infection also occurs in South West Africa, Bechuanaland, Angola and Belgian Congo.

It will be noticed from the available records (Table (I)) that only a single case of equine besnoitiosis is recorded from Europe, that it appeared as an important malady in Sudanese horses, that it occurs sporadically in the Union of South Africa with its large equine population, and that it seems to be absent from the remaining African territories in which bovine besnoitiosis occurs enzootically. Its apparent absence may be attributed to the incidence of horsesickness in Angola, Bechuanaland, Belgian Congo and Ovamboland, as reflected by the extremely low equine population.

Up to the present murine besnoitiosis has only been recorded in a white-footed mouse from the United States of America.

T A B L E I

Continent:	Country	:Animal:	Reference.
Europe	:Southern France	:Cattle:	Cadéac, 1884; Besnoit
			:and Robin, 1912,1913,
			:1914. Cuillé, Chelle
			:and Beruleau, 1936;
			:Cuillé and Chelle,1937;
			:Barrairon, 1938.
	:Northern France	:Horse	:Henry and Masson, 1922.
	:(Rheims)		
	:Portugal (Alentejo)	:Cattle:	Franco and Borges,1916;
	:Portugal (Imported)	: "	:Leitao, 1949.
	:cattle from Angola)		
Africa	:Angola (Alvito, Beja,	:Cattle:	Leitao, 1949.
	:Elvas, Evora,		
	:Grandola, Moura etc.)		
	:Bechuanaland	:Cattle:	Director of Veterinary
	:(Serowe)		:Services, 1957.
	:Belgian Congo	:Cattle:	Colback, 1951; Herin,
	:(Ruanda-Urundi)		:1952.
	:South West Africa	:Cattle:	De Boom, 1947.
	:(Ovamboland)		
	:Sudan	:Horses:	Bennett, 1927 - 1939;
			:Going, 1927.
	:Sudan (Nuba Mountains	: "	:Williams, 1932.
	:in Southern Kordofan)		
	:Sudan	:Cattle:	Bennett, 1928.
	:Union of South Africa		
	:Transvaal districts		
	:Lydenburg	:Cattle:	Meeser, 1954.
	:Marico	: "	:Thomas, 1950;Schulz 1951.
	:Pietersburg	: "	:Van der Merwe, 1953.
	:Potgietersrust	: "	:Herter, 1950.
	:Pretoria	: "	:Van der Vyver,1945;
			:De Wet, 1950;Pols,1953.

Table I cont. /9.....

TABLE I (Cont.)

Continent:	Country	Animal:	Reference.	
Africa (cont.)	Union of South Africa			
	Rustenburg	Cattle	Hofmeyer, 1945; Thomas, Freaan and Neitz, 1943; Louw, 1954	
		Horse	Thomas, 1950.	
	Soutpansberg	Cattle	Thomas, 1950; Mansvelt, 1953.	
	Waterberg	Cattle	Thomas, 1943.	
		Horse	Thomas and Adelaar, 1947.	
	Eastern Cape Province District.			
	Grahamstown	Horse	Schulz and Thorburn, 1941, 1955.	
	Natal Districts			
	Umvolozi (Empangeni)	Horses	De Kock, 1947.	
	Vryheid	Horses	Lamprecht, 1950.	
	United States of America	Hamilton in Montana	White-footed mouse	Frenkel, 1953, b.

AETIOLOGY

Besnoitia besnoiti. (Marotel, 1912).

Synonyms:- Sarcocystis besnoiti Marotel, 1912.

Gastrocystis besnoiti (Marotel, 1912).

Globidium besnoiti (Marotel, 1912).

(a) Morphology: Fairly comprehensive accounts of the histological studies on the causal agent of bovine besnoitiosis, accompanied by a series of microphotographs, have been given by Besnoit and Robin (1912) and Franco and Borges (1916). Although the descriptions were limited to a study of advanced stages of the development of the organism, they nevertheless served as a basis for later investigations on B. bennetti Babudieri, 1931, by Bennett (1933), B. jellisoni Frenkel, 1953, by Frenkel (1953 a, 1954) and B. besnoiti (Marotel, 1912) by Pols, 1954 a, b).

/10.....

It will be seen from the description, given below, that the advances made in the research resulted from the discovery of laboratory animals susceptible to the Besnoitia spp., and the availability of improved staining techniques. It has thus become possible not only to supplement the original observations but also to modify certain views held by previous investigators. The progress made comprises the determination of the vertebrate life-cycle of the infectious agent, and the origin and development of the cystic wall as well as the identification of certain chemical components of the parasite, and cystic wall as revealed by the staining reactions.

The present account on the morphology of B. besnoiti is based chiefly on the observations made on rabbits used for the serial passage of this parasite. Recovery, that followed, the artificial infection, made it possible to trace the development and behaviour of the parasite in the circulating blood and tissues, and to observe the response of the host to the infectious agent during the period of the febrile reaction and the ensuing chronic phase of the disease. Observations recorded by Pols (1954 a,b) will be recapitulated, and extended to include the studies on the origin and development of the cystic wall which, up to the present, had not been fully understood. Observations made on rabbits will be compared with those made on cattle and a single goat that developed a typical but non-fatal besnoitiosis reaction.

The forms of the organism circulating in the bloodstream and seen in the blood, lung and testes smears of rabbits vary in size from 5 - 9 microns in length and from 2 - 5 microns in width. Similarly there is a variation in shape. Oval forms slightly pointed at one end are the most common in blood smears. Curved forms with rounded ends (banana-shaped) and crescentic types are found more rarely except in lungs and testis smears where they are quite common. They are also more frequent in smears obtained late during the temperature reaction, when the number of monocytes is decreasing. They give the impression of being more shrunken or mechanically compressed. Only a few round forms were observed.

/11.....

With Gram stain the trophozoites gives a negative reaction, and stands out clearly when stained with Moeller's method: a distinct blue on a reddish background, but internal structural detail is by no means distinct. With Giemsa stain the cytoplasm is granular and stains blue, usually slightly lighter at the one (blunt) end. At this end one often discerns a yellowish red "caplike" area from which fine striations radiate toward the nucleus. Occasionally darker blue granules as well as vacuoles are noticed. They are distributed throughout the cytoplasm, but more towards the pointed end. With Nile blue sulphate and Periodic-Acid-Schiff reagent they react positively; pretreatment with saliva abolishes the latter reaction. It is, therefore, concluded that the cytoplasm contains glycogen. (Interestingly enough decolourisation of previously P.A.S. stained preparations, and subsequent treatment with saliva does not abolish the P.A.S. reaction). Other staining reactions were performed on sections of skin. The results will be mentioned when dealing with the cyst forms. The banana- and sickle-shaped forms, by nature of their denser cytoplasmic structure, stain more intensely and detail is less distinct.

The nucleus is situated near the centre. It may, however, occur in various positions and does not conform to a definite pattern or size. In the oval shaped parasite it varies from a fairly compact form to a loose network of chromatin. With Giemsa it stains the usual reddish purple, darker when the nucleus is more compact. The latter appearance is the one seen in the crescent-shaped organisms as a rule. The oval form may be binucleate. On application of the Feulgen technique, the nuclei take on a very faint tinge of purple, in sharp contrast to the brilliant reaction given by the nuclei of the leucocytes.

The intracellular parasites do not differ from the extracellular ones except that in blood smears the banana- and sickle-shaped forms are often not as frequent. As can be expected extracellular parasites are difficult to discern in sections, and the intracellular ones appear much smaller and more crescent-shaped. Great numbers of

/12.....

divisional forms are seen in different stages of development, both intra- and extracellularly. The most common of these is a binucleate one where division of the cytoplasm is in the process of taking place or has just been completed. Otherwise two parasites may be seen closely approximated as to suggest early separation. Multiple divisions do occur, such are exemplified by cytoplasmic masses containing 4 to 8 nuclei more or less regularly spaced.

In skin biopsies and organ specimens taken at autopsy, sectioned singly and serially and stained with haemalum-eosin, organism after much searching, could be seen lying both, singly and in groups, between the connective tissue fibres. It is impossible to state whether they were in the lymphatics or not. Infrequently organisms are also seen in wandering or fixed histiocytes, either singly, in pairs or in larger groups in a vacuole in the host cell. As stated above such organisms in section appear in a much contracted form; they measure approximately 3 microns in length and 1.5 microns in width. Depending upon the number of parasites in them, vacuoles may be quite small, measuring approximately 8 microns in diameter during the third week after infection of the subject. In specimens taken at progressively longer intervals after infection they become much larger; eventually at about nine weeks they range from 80 to 120 microns in size with extremes of 50 to 200 microns. They then contain a correspondingly increased number of organisms. These are laden with glycogen as may be inferred from the brilliant P.A.S. reaction which is abolished after treatment, with saliva leaving only a cytoplasmic hull. With Hale's stain the cytoplasmic contents react almost equally well indicating the presence of acidmucopolysaccharide. The latter tends to be concentrated towards the blunt end. However, the quantity present in any one individual organism varies from virtually nothing to relatively large accumulations in contrast to the glycogen which is virtually always present. The constant positive reaction to silver impregnation, performed according to the Gömöri technique, could also be ascribed to this fact. As in the case of the blood parasites, the nuclei react very faintly indeed to Feulgen's staining method. Only by employing Wratten's filters, could any satisfaction be obtained that there really was a purplish colour.

When one or a few organisms occupy the vacuole there is a clear space around the parasites. When present in larger numbers they are more tightly packed, leaving little or no intervening clear areas. In large vacuoles the outer edge forms a distinct membrane which has been referred to as the inner membrane (Pols 1954 b).

The infected host cell is seen to contain two or more nuclei. These are very vesicular with a delicate nuclear membrane and numerous enlarged nucleoli. In distinct contrast to the nuclei of the organism, they are clearly Feulgen positive, although their vesicular nature makes for a scant distribution of positive material, distributed just under the nuclear membrane and here and there towards the centre, as discrete rounded knots. The nucleoli are Feulgen negative with occasionally a narrow sickle-shaped positive edge. Because of the vesicular nature of these nuclei, their staining reaction is somewhat dulled when seen against the green background of underlying Feulgen negative cytoplasm. The nuclei increase vastly in number and generally form a radiating crown at some distance from the periphery of the cell. Mitoses are observed infrequently. When division occurs, all nuclei present in one cell, divide simultaneously. Ultimately, due to parasitic encroachment, the nuclei become wrinkled, the nuclear membrane folded and the whole nucleus elongated. Finally they become pycnotic and compressed to a very narrow elongated strip of nuclear material. The cytoplasm of the host cell enlarges to gigantic proportions, becomes deeply basophilic and markedly granular.

Between the nuclei and the periphery of the cell it is somewhat lighter, and here large distinct absolutely clear vacuoles are seen in embedded sections. Some of these vacuoles may actually be interpalated between the nuclei. In this area too, vacuoles containing parasites occur. The centre of the host cell shows an enormously enlarged spherical centre which is much less basophilic. In it frequently the very extensive negative image of a Golgi apparatus may be seen. The outline of this central area is often accentuated by a pallisade of dark basophilic cytoplasmic streaks lying around it. They may be shown up well in iron haematoxylin preparations.

In the earlier phases of the host cell's development its cytoplasm gives only a light P.A.S. positive reaction. When it has attained its maximum development large granules of intensely red staining material are scattered throughout the central zone, tightly packed in large masses immediately around it and between the nuclei, whereas the peripheral area is relatively clear. The reaction is abolished by pretreatment with saliva. As the parasite enlarges the P.A.S. reaction is lost, although the rate of loss does not appear to be constant. At the cell membrane there is a distinct P.A.S. positive zone, which soon merges with the rest of the capsule; its exact situation whether intra- or extracellular is very difficult to determine. The latter interpretation is probably correct. The positive reaction remains despite salivary digestion. Despite the strong P.A.S. reaction exhibited by the cytoplasm of the host cell during its phase of maximal development it was never found to react to Hale's stain for acidmucopolysacharide. The central zone of the host cell's cytoplasm is also argyrophilic. The same granular appearances as described for the P.A.S. reaction is seen after Gömöri's reticulum impregnation. Later the argyrophilic granules condense at the periphery of the central zone. With development of the parasite the argyrophilic granules are pushed aside in irregular masses and eventually the argyrophilia disappears.

With enlargement of the parasite containing vacuoles, the regularity of the host cell structure is disturbed, as already will have been inferred from the foregoing description of the various staining reactions. When packed with organisms the cytoplasm is compressed to a very narrow rim ("the intermediate membrane"), and between this and the inner membrane a varying number of compressed host cell nuclei may be seen. Sometimes the occurrence of organisms in different vacuoles in one host cell, produce a bi- or tri-ocular appearance. No nuclei appear in the septa.

During the development and regression of the host cell the following sequence of events is observed around it. At first it becomes surrounded by a homogenous matrix, around which young collagenous fibres are laid down. The matrix stains poorly and gives a negative reaction with

P.A.S. and van Gieson, and stains a light blue with Hale's stain. In it delicate fibres, argyrophylic, fuchsinophilic, P.A.S. and Hale positive are seen to course. It is probably a condensation of these fibres which are responsible for the narrow rim of P.A.S. and Hale positive, argyrophilic material demarcating the boundary between the future capsule and the host cell, and already referred to when dealing with the P.A.S. reaction of the latter's cytoplasm. It must be mentioned, however, that the argyrophilia was not a constant feature.

With enlargement of the host cell the capsular zone becomes progressively more and more positive to the P.A.S. reaction, and at the same time stains intensely with Hale's stain. With the latter stain a thick deep blue capsule, with a very narrow lighter staining inner rim, then surrounds the host cell. As the P.A.S. reaction gains in intensity so does the capsule become more and more fuchsinophilic. At the same time the intensity of the Hale reaction decreases, and the whole process goes hand in hand with the deposition of collagenous fibres. In developing cysts these fibres can be traced, distinctly running from the surrounding connective tissue. Fibroblasts accumulate more or less regularly around this collagenous mass. Eventually the collagenous fibres become hyalinised, intensely fuchsinophilic and P.A.S. positive. Especially with the latter reaction the inner part of the cyst wall is intensely coloured. Whereas in the earliest stages of cyst development salivary predigestion affects the subsequent P.A.S. reaction but slightly, it produces quite a pronounced effect in the fully developed cyst. Despite this, a considerable amount of P.A.S. positive material remains especially towards the inner part of the cyst wall. In this stage Hale's stain produces only narrow complete or incomplete ringshaped zones or granular accumulations of blue staining material. Where capsules abut on each other similar material is found in considerable quantity in the intervening spaces. Argyrophilic fibres become less pronounced as the cyst approaches maturity.

/16.....

In cattle B. besnoiti presents essentially similar morphological features. The circulating organisms of which only very few were seen, did not differ in any way. The fully formed cyst in cattle is much larger than that found in the rabbit; measuring up to 600 microns in diameter. When seen singly they are spherical or sometimes slightly spheroidal. When present in large numbers they may be compressed; the outer membrane or cyst wall then becomes confluent. Organisms expressed from the cysts and examined in smears are mostly crescentic and measure on an average 2x7 microns. In saline suspension they exhibit moderate motility by flexing and slightly contracting and then extending. The parasite seen in the goat did not differ from that seen in cattle. Around fully formed cysts no further tissue reaction is elicited. In infections of long standing they appear as perfectly bland parasites with little indication of their genesis. In cattle an occasional degenerating cyst was observed with remnants of the now incomplete capsule and the central necrotic and calcifying debris, with a localised tissue reaction indicating resorption.

(b) Multiplication:- B. besnoiti, like B. jellisoni, multiplies within the host cells by repeated binary fission (Frenkel, 1953 a; Pols, 1954 a, b). Multiple divisions do occur, but when compared with the former, they are so rarely observed as to lead one to suspect that they are aberrant forms (Pols, loc. cit). Reproduction continues within monocytes and possibly also in neutrophils until they rupture, and is then again resumed after parasitic invasion of new host cells. In the hypertrophied histiocytes multiplication persists until the resulting cyst, with its firm capsule, is filled to capacity. In this situation parasites remain dormant for periods of up to ten years, after a natural infection (vide infra - Immunity). Absence of any degenerative changes of cysts during this long period, as determined histologically, suggests that the parasites survive in chronically affected cattle for even longer periods.

/17.....

(c) Habitat:- Bennet (1928) described the presence of Besnoitia parasites, initially termed "sarcospores" and later, after Wenyon's identification, "Globidium spores" in large numbers in blood smears obtained by skin punctures from affected horses. On taking blood from the jugular vein, for the preparation of smears, parasites could not be found. He concluded that probably accidental contamination from incised cutaneous cysts had occurred. In view of the fact that blood is only infective during the primary stage of the disease (Cuillé, Chelle and Berlureau, 1936; Pols, 1954 a), and Bennett's cases seemed to be advanced, his final conclusion is most likely to be correct. Cuillé and Chelle (1937) spoke of a "virus" in the blood of cattle during the primary stage of the disease, and tried to determine whether this infectious agent was attached to the erythrocytes or free in the plasma, but without success. It may thus be presumed that B. besnoiti had not been observed previously in cattle in any form other than that in cysts.

In order to determine the habitat of the parasite seven head of cattle, listed in Table A of the appendix, were subjected to daily blood and lymphnode smear examinations. In four of these cases extracellular parasites were seen in blood and lymphnode smears, on one day only, during, or at the most two days after the height of the febrile reaction. In one animal they appeared in the blood and lymphnode smears, in two cases in blood smears, and in one in a lymphnode smear only. One or at the most three parasites could be found per smear. Parasites in the circulating blood and lymphatic tissues of cattle are thus extremely rare. The appearance of parasites in cysts situated in the skin and adjacent connective tissue has already been commented upon (vide supra - Morphology).

In contradistinction to cattle, the rabbit forms a far better subject for the study on the habitat of the parasite. In 80 per cent of the 440 rabbits, used for the 125 serial passages, parasites could be demonstrated in blood smears. It may be interesting to note that from generation 119 to 125 the percentage of positive smears dropped to about 60 per cent.

In rabbits parasites were demonstrable in the circulating blood for either one or for six consecutive days, from about the peak of the thermal reaction or shortly thereafter. The average period was about two days. It is possible that in some cases the microscopic infection may have been longer since rabbits with the best reactions were selected, as a rule, as donors and were killed for the collection of blood.

For the demonstration of parasites smears were prepared by pushing the upper slide, and drawing the drop of blood behind it. In this way the blood formed a flare at the end of which the majority of leucocytes collected. An increase in monocytes, best seen here, presaged the presence of parasites within the next few days. This site, too, is the most favourable for finding parasites. In the average positive smears one can expect one trophozoite per four or five microscopic fields. As a rule the distribution is irregular. The majority of parasites in blood smears were extracellular. Intracellular parasites in monocytes occurred less frequently. Organisms were encountered occasionally in neutrophiles.

After death smears were made from the lung, liver spleen and testis from a relatively large number of rabbits, and also from the brain, kidney, lymphnodes, subcutaneous oedematous fluid and faeces in some cases. Provided the period intervening between the height of the thermal reaction and organ smear examination did not exceed ten to twelve days, parasites could be demonstrated in lung and testis smears, rarely in those of the liver and spleen, while all the remaining preparations proved to be negative. On some occasions lung and/or testis smears were positive when no parasites could be found in blood smears.

Although fully aware of the naturally occurring polymorphism of Besnoitia parasites, and of possible artefacts arising during the preparation of smears, the writer considers the increased number of so-called contracted forms, i.e. banana- and sickle-shaped types, during the later stages of the febrile phase of the disease as significant. They are those forms which are subsequently seen in the interstices of cutaneous and subcutaneous connective tissue. Here they enter the tissue

/19.....

histiocytes which either have undergone reactive changes just prior to penetration or react soon thereafter. The reaction of the host cell is evidenced by multiplication of the host cell nucleus, enlargement of its cytoplasmic mass, i.e. the formation of a plasmodium, and the appearance of a distinct clear vacuole in which the parasite is seen to lie. More than one of such parasitized vacuoles may develop. The parasite proliferates by binary fission and, at the same time, the vacuole enlarges. Meanwhile cytoplasmic enlargement, and nuclear multiplication of the host cell continues until its body attains gigantic proportions. Large oval vesicular nuclei with prominent nucleoli are disposed, shingle fashion, around the periphery in a zone of more intensely basophilic cytoplasm. Towards a centrally situated, spherical well demarcated zone, this basophilic material is concentrated in frequently distinct radially disposed rod-like structures. The large central zone, which stains lighter, is interpreted as an hypertrophied centrosome in which canaliculi of an equally enlarged Golgi apparatus can be distinguished as a negative image in ordinary preparations. The enlarging vacuoles with parasites, at this stage, still occupy a segment or segments of the peripheral zone in between the host cell nuclei. At this stage the parasites are still surrounded by a clear rim. During subsequent enlargement they come to fill the vacuole completely and lie tightly packed. As a result the host cell's cytoplasm and nuclei become crowded towards one end. When two or more vacuoles are present in the same cell, their opposed sides are compressed into a thin septum containing no nuclei, although the latter may extend towards the septum where the opposed edges of the vacuole diverge. Eventually cytoplasm and nuclei are reduced to a very narrow, dark staining rim, the former only visible in fortunate sections of the cyst. During this process young connective tissue fibres are laid down around the host cell by fibroblasts in the vicinity. These fibrils collagenize, and eventually hyalinize to form the well known, thick, more or less homogenous cyst wall.

The histo-chemical reactions performed indicate that there is a temporary development of polysaccharide material in the host cell during the earlier phase of parasitism, especially in the interzonal region near the periphery of the greatly enlarged centrosome. According to the Hale reaction this is not acidmucopolysaccharide. The

vesiculation and chromatin margination of the host cell nuclei is clearly revealed by the Feulgen reaction.

The pericellular connective tissue reaction yields a histochemical response which, in all respects, is similar to that of fibrous connective tissue formation. Young argyrophilic fibrils condense against the cyst wall. They are strongly positive for acidmucopolysacharides. In the surrounding matrix further fibrils are laid down, strongly positive for the same substance. Further laying down of fibrils gives the forming cyst wall a concentric effect. A process of collagenization then sets in, the argyrophilia and reaction for acidmucopolysacharides decreases but at the same time the mucopolysacharides and glycogen content increases, the accumulation being greater in the older i.e. inner part of the cyst wall. Eventually this part remains strongly P.A.S. positive, even after diastase treatment, indicating probably convergence of glycogen into mucopoly-sacharide.

It is thus quite clear that the cyst in B. besnoiti is host formed. There is no reason whatsoever to accept a parasitic origin of the cyst wall merely on grounds of its argyrophilia or of its P.A.S. positive reaction. At most the residual mucopolysacharide content, concentrated along the inner part of the cyst wall, indicates a reaction to the presence of the parasite. The clearly demonstrable fibrils of connective tissue converging around the parasitized cell offer definite histological proof. Also it is inconceivable that parasitic material could produce a cyst wall through the intervening medium of the host cell cytoplasm.

According to Frenkel and Friedlander (1951) Weinman was probably the first to use "pseudocyst" as a noncommittal descriptive term since there is no proof that the cyst wall was elaborated by the organism. Frenkel and Friedlander (loc. cit.) to whom it appeared that the pseudocystwall of Toxoplasma was likely to be derived from the micro-organism, employed the term cyst or pseudocyst interchangeable. In order not to confuse matters by an introduction of new terms, the author proposes that the term pseudocyst be restricted to those cyst-like structures formed by the host under the influence of the parasite. In this sense Jacobs (1956) employed the term with reference to Toxoplasma, although he contrasted the latter to Besnoitia and Sarcocystis.

The above description can be briefly summarized as follows:- After the artificial infection of a susceptible host, and lapse of a due incubation period a hyperthermia persisting for several days, is observed. During the period of the febrile reaction parasites appear in the blood stream in small numbers in the case of cattle, and in relatively large numbers in rabbits. Simultaneously they are demonstrable in the superficial lymphnodes of cattle, and in the subcutaneous oedematous fluid, lung, testis, liver and spleen of rabbits, either by means of microscopic smear examination or subinoculation tests. Based on the relative numbers of organisms found, observations indicate that they appear primarily in the blood circulation and probably in the lymph stream. Here they occur extracellularly in more or less oval forms, but more acutely rounded at one end. It is considered that these forms invade the greatly increased numbers of monocytes, where they undergo multiplication, eventually to be set free into the circulation again. It must be stressed that intracellular proliferating forms lie in the cytoplasm of the host cell unincapsulated by any structure. They are not seen inside a distinct vacuole. A number of forms in which the cytoplasm is in various stages of longitudinal cleavage, subsequent to nuclear division, are seen extracellularly in air-dried smears. Despite the care with which these smears are prepared, the relative scarcity of such forms leads one to consider them as divisional stages mechanically freed as a result of cell rupture consequent upon drawing the smear. It is not possible to state whether or not the host cell undergoes degeneration and dissolution in the process of parasite liberation.

After cessation of the fever the parasites disappear from the blood circulation, and reappear in actively growing histiocytes situated in the fibrous tissue of the cutis, subcutis, aponeuroses, testis, sclera and mucous membrane of the upper respiratory tract. In the latter two sites numerous cysts can be detected readily with the naked eye. The host cell becomes markedly hypertrophied, and shows a variable number of nuclei which multiply by binary fission. The parasites create a vacuole, reproduce

/22.....

rapidly and push the nuclei to the periphery of the host cell. Cysts reach maturity approximately six to eight weeks after an artificial infection.

(d) Life-cycle:- Within the vertebrate host B. besnoiti shows an asexual reproduction in monocytes, histiocytes and possibly also in neutrophils. The natural mode of transmission of besnoitiosis is unknown. In the event that it should be found that an arthropod serves as a biological transmitter, it is possible that a sexual phase in the invertebrate life-cycle may be disclosed.

(e) Cultivation:- No attempts have been made to grow B. besnoiti in chick embryos and tissue cultures.

(f) Resistance to physical and chemical agents:- For artificial transmission experimental knowledge about the duration of the viability period of an infectious agent is of practical importance. At the time when these investigations were undertaken the only available records on the resistance of B. besnoiti to the abovementioned agents were those of Cuillé, Chelle and Berlureau (1936) and Cuillé and Chelle (1937). For their tests they employed either bovine blood collected during the febrile phase of the disease or cutaneous cysts derived from a chronic case of besnoitiosis. The recipients were cattle obtained from a non-enzootic bovine besnoitiosis area in France. Fresh blood was administered within a few minutes after its withdrawal while citrated blood was stored at room temperature for either 5 or 24 hours before injection. The preparation of the cyst suspension involved removing an adequate piece of skin, grinding it in a mincing machine, and preparing a suspension in "physiological serum". These manipulations required six hours. The various inocula were administered intravenously. The experimental observations are listed in the subjoined Table II.

In interpreting their results Cuillé et al. (loc.cit.) assumed that a typical reaction in a recipient served as a criterium for viability of the infectious agent, and concluded that the survival period of B. besnoiti in citrated blood was five hours. A possible development

/23.....

of a mild or an inapparent infection in the test animals was not taken into account, and consequently neither immunity tests nor histological examination of skin sections were undertaken. It will be seen from Table II that in one of the two recipients that received one litre of citrated blood, stored for 24 hours at room temperature a "bout of fever" appeared 15 days after the injection, while the other animal failed to show any clinical symptoms. A similar observation was made on the two recipients that obtained the suspension of emulsified cysts. One animal developed a "bout of fever" fifteen days later, while the other showed no symptoms.

The writer is of the opinion that Cuillé et al. (loc. cit.) were not justified in concluding that the infectious agent failed to remain alive for periods longer than five hours. This assertion is based on his own experience (Pols, 1954). In these tests two oxen each received one litre of blood collected from two donors which were showing fever. One of the recipients developed a thermal reaction six days later, and the other no clinical symptoms at all. Histological examination of skin section prepared three weeks later, revealed typical Besnoitia cysts in both animals. Consideration of these results suggests that, in the tests conducted by Cuillé et al. (loc. cit.), the infectious agent survived for periods longer than five hours, and that they were probably the first investigators to effect transmission with parasites derived from cysts harboured by a chronically affected animal.

For the present studies on the duration of the viability period of B. besnoiti in vitro, rabbit blood showing a microscopic infection was employed. The different chemical agents, the various temperatures and the periods to which the infectious agent was exposed, and the rabbits used for determining the fate of the parasite are listed in the appended Table II and in Table A of the Appendix. For these tests either 4.0 or 5.0 ml of blood administered by the intraperitoneal route, and in one instance 10.0 ml injected subcutaneously were employed as

/24.....

inocula. Rabbits used for the serial passage of the strain were used as donors, while the recipients acted as controls. The latter animals all reacted promptly to besnoitiosis after the intraperitoneal injection of fresh citrated blood. In some instances where recipients failed to react to the treated blood, immunity tests were performed in order to exclude the possibility of an in-apparent infection.

It will be noticed from the tests listed in Table III that exposure of infective blood to either potassium oxalate or heparin for a period of 30 minutes at room temperature had no influence on B. besnoiti. Edington's solution commonly used as a preservative of viruses, had no immediate action but it killed the organism within 90 minutes after exposure. At room temperature the infectious agent in sodium citrate remained potent for periods of up to 48 hours but not after 96 hours. In citrated blood stored for 24 hours at 37°C the organism died. Blood held at +4°C for either 72 or 96 hours remained active. The end point of the viability period of B. besnoiti in citrated blood maintained at +4°C still needs to be determined. Further tests showed that the parasite did not survive when stored at -76°C for 12 days, and for 24 and 30 months and at -18°C for 30 days.

The addition of penicillin and streptomycin to citrated blood did not improve the keeping qualities of the infectious agent. On the contrary comparisons between the duration of the viability periods of the organism in citrated blood with that in citrated blood to which the two abovementioned antibiotics had been added, indicated that the latter products had a detrimental influence on vitality. When held at 20°C for 48 hours, and at +4°C for 96 hours the organism died but when maintained at +4°C for 72 hours it remained alive. These results suggest that the detrimental influence of the antibiotics is retarded at a low temperature of +4°C, and hastened at a higher temperature of 20°C.

Consideration of these results shows that the duration of the survival periods of B. besnoiti as determined by the present tests is very much longer as ascertained previously by Cuillé et al (loc.cit.). It has

RESISTANCE OF *B. besnoiti* TO PHYSICAL AND CHEMICAL AGENTS.

No. of Rabbits.	S t o r e d   B l o o d			No. that reacted.	No. that died.
	Time	Temperature °C	Anticoagulent		
8	0	39.0	Sodium citrate	8	3
2	1.5 hours	20.0	" "	2	0
2	24 hours	20.0	" "	2	0
1	24 hours	37.0	" "	0	0
1	48 hours	20.0	" "	1	0
1	72 hours	+4.0	" "	1	1
1	96 hours	20.0	" "	0	0
1	96 hours	96.0	" "	1	1
2	30 days	-18.0	" "	0	0
2	12 days	-76.0	" "	0	0
2	24 months	-76.0	" "	0	0
2	26 months	-76.0	" "	0	0
1	30 months	-76.0	" "	0	0
2	0	39.0	Sodium citrate together with <sup>x</sup> penicillin and <sup>x</sup> streptomycin	2	1
2	48 hours	20.0	Sodium citrate together with <sup>x</sup> penicillin and <sup>x</sup> streptomycin	0	0
1	72 hours	+4.0	Sodium citrate together with <sup>x</sup> penicillin and <sup>x</sup> streptomycin	1	1
1	96 hours	+4.0	Sodium citrate together with <sup>x</sup> penicillin and <sup>x</sup> streptomycin	0	0
1	0.5 hours	20.0	Potassium oxalate	1	1
1	0.5 hours	20.0	Heparin	1	1
2	0	39.0	<sup>x</sup> Edington's fluid	2	1
2	1.5 hours	20.0	" "	0	0

<sup>x</sup> See Table A of Appendix

also become apparent that rabbits are better suited for these tests than cattle, but that the determination of end points of the viability periods in different circumstances would entail the use of an extremely large number of animals over a long period. The limited time available did not permit conducting detailed systematic investigations, and hence studies were confined in establishing a practical and reliable method for the preservation of the infectious agent in vitro. The fact that B. besnoiti in citrated blood survives for four days at a temperature of  $+4^{\circ}\text{C}$ , makes it possible to submit viable parasites from any part of the enzootic area in Transvaal to the central laboratory at Onderstepoort for biological studies.

(g) Biological properties:- Besnoit and Robin (1912) established that B. besnoiti spores contain a toxin highly fatal for rabbits but to which guinea pigs and rats are refractory.

Whether or not immunologically different strains occur in nature still needs to be determined. Frenkel (1953 a), who tested sera from naturally infected cattle from South Africa, found that there is no measurable antibody content using B. jellisoni as antigen in the dye-test developed by Sabin and Feldman (1948).

(h) Maintenance of the B. besnoiti strain:- The successful transmission of B. besnoiti to rabbits by means of blood, derived from an affected ox during the febrile stage of the disease, offered a convenient method for maintaining the "Fuls" strain under laboratory conditions (Pols, 1954 a, b). During the course of the 125 serial passages it became apparent that several breeds of rabbits and that both sexes are equally susceptible. Cardiac blood from rabbits, when blood smears of donors showed a microscopic infection was used as inoculum. On few occasions blood from the marginal ear vein was employed.

During the first four generations the inoculum was administered by either the intravenous (one case, 3.0 ml), intraperitoneal (three cases, 2.5, 3.0, 5.0 ml) or subcutaneous route (three cases, 2.5, 10.0, 10.0 ml).

The ensuing reactions were characterised by a marked variation in the duration of the incubation periods. In the former rabbit it was six days, in the next three it varied from eight to twelve days, and in the latter three recipients it varied from thirteen to sixteen days.

From the fifth generation onwards the intraperitoneal route was employed as the standard method. The dose of blood used varied from 2.0 to 6.0 ml. Although no difference in the type of reactions followed this modification, a standard dose of 5.0 ml of citrated blood was used from generation 25 onwards.

A total of 440 rabbits were employed for the 125 serial passages. Of these 11 animals died from causes other than besnoitiosis, 352 developed typical reactions accompanied by microscopically demonstrable parasites, 68 only showed hyperthermia and/or subcutaneous swellings, and 9 failed to reveal any symptoms. Neither subinoculations nor immunity tests were undertaken in the latter group of rabbits, and hence it is impossible to state whether or not an inapparent infection had occurred. Consideration of these observations makes it apparent that 20 per cent of recipients could not be relied upon as competent reservoirs for the maintenance of the "Fuls" strain, and that at least three rabbits are required for each serial passage.

(i) Classification:- The names assigned to various species of Protozoa of the elementary tract of the horse, domestic ruminants, armadillos, marsupials and certain snakes included in either the genera Eimeria Schneider 1875, Sarcocystis Ray Lankester 1882, Globidium Flesch 1883, Balbiania Blanchard 1885, Gastrocystis Chatton 1910, Ileocystis Gilruth and Bull 1912, Lymphocystis Gilruth and Bull 1912 or Haplogastrocystis Chatton 1912, of those occurring in the cutis, subcutis, aponeuroses, sclera and mucous membrane of the upper respiratory tract of cattle and horses included in either the genera Sarcocystis, Globidium or Besnoitia Henry 1913, and of the one found in the periosteum and tendons of the reindeer and the other encountered in the connective tissue of organs of the opossum included in the genus Fibrocystis Hadwen 1922,

have long been subject of discussion, much of which hopelessly intermixed zoological and nomenclatorial considerations.

Before discussing the classification of the parasites responsible for besnoitiosis, it will be necessary to give a brief historical review about the investigations on the abovementioned micro-organisms during the last twelve decades. In doing so it is hoped that the reader will gain a clearer concept of the events that lead to the confusion in the nomenclature, and of the investigations that eventually permitted the correct identification of the Besnoitia spp. responsible for bovine, equine and murine besnoitiosis. Recent investigations by Frenkel (1953 a, 1955) and Pols (1954 a, b) have shown that the genus Besnoitia Henry 1913, is morphologically closely related to the genus Toxoplasma Nicolle and Manceaux 1909. In fact the present investigations have shown that the microscopic identification of the two genera is based chiefly upon the recognition of the host cells. Members of the genus Besnoitia are harboured temporarily by monocytes, and permanently by histiocytes, while the heteroxenous Toxoplasma sp. chiefly parasitizes cells of the reticulo - endothelial system, macrophages, monocytes and endothelial cells.

Miescher (1843) discovered the Sarcosporidia in the muscle fibres of a mouse which Kühn (1865) named Synchitrium miescherianum. As this organism evidently did not belong to this genus Ray Lankester (1882) included it in the genus Sarcocystis, while Blanchard (1885) named it Sarcocystis muris. Th. Smith (1893) found cysts, varying from 300 to 400 microns in diameter, which contained numerous merozoites in the mucous membrane of the small intestine of cattle in the United States of America. He concluded that the parasite was probably related to the Sarcosporidia. Marotel (1907), who found similar structures in the intestine of cattle in France, suggested that they represented the schizogonous phase of Eimeria zürni. (Rivolta, 1878). Bugge (1910) encountered pin-head sized cysts in the intestine of a calf in Germany, and suggested that they were immature stages of either coccidia or gregarines.

/28.....

Besnoit and Robin (1912) discovered a parasite in the skin of cattle from the Pyrenees. It was named Sarcocystis besnoiti by Marotel (1912). The following year Henry (1913) created the genus Besnoitia and referred to the cutaneous parasite as Besnoitia besnoiti (Marotel 1912). Nüller (1920 revised the generic nomenclature of the genera Globidium, Gastrocystis, Ileocystis, Lymphocystis, Haplogastrocystis and Besnoitia. He retained the genus Globidium, and rejected the remaining generic names on grounds that they did not possess any distinct characteristic morphological features. He concluded without justification, that the cyst-like structure found in the intestine of cattle is identical with the cutaneous form, and included it under the name Globidium besnoiti (Marotel 1912).

Müller (1914) encountered cysts varying from 120 to 300 microns in size harbouring numerous spores in the intestine of cattle from Germany. He considered these structures to be schizonts of a coccidia. Railliet (1919) who revised the literature dealing with intestinal cysts, proposed that the parasite be named Gastrocystis smithi as there was no evidence that it was related to E. zürnii. Bruce (1921) demonstrated cysts in the small intestine of a calf in Canada. In one of the cysts he found merozoites, and concluded that these bodies were schizonts. In Holland Van Nederveen (1921) saw globular structures in the intestine of a calf which he identified as Gastrocystis smithi Railliet 1919. In England Pillers (1928) encountered cysts in the intestine of a bull which he named Globidium smithi. Hassan (1935) described cysts of up to 1000 microns in size containing numerous spores 13.0 microns in length, and from 2.0 to 2.5 microns in width in the mucous membrane of the pyloric end of the abomasum, and in that of the ileum of Indian cattle. As the parasitic cysts had not been described previously in the abomasum of cattle, he considered this parasite to be a new species, and proposed the name Globidium fusiformis. In the United States of America Boughton (1942) observed that cysts in the mucosa of the small intestine of cattle occurred in association with coccidial infections, especially those of Eimeria bovis Züblin 1908. He also demonstrated the appearance of E. bovis oocysts five days after feeding material containing cyst-like structures to a week-old calf. From these observations he concluded that the cyst-like bodies were schizonts of one or more species of bovine coccidia, E. bovis being the most likely in the material observed by him.

These investigations were followed by well planned biological and histological studies of Hammond, Bowman, Davis and Simms (1946) who confirmed the conclusions of Boughton (loc. cit.). They transmitted E. bovis to a series of calves, and showed that the schizonts developed in the small intestine, while the sexual phase was usually found in the caecum and colon except in severe infections. In these circumstances sexual stages also appeared in the distal end of the small intestine.

The work conducted by Boughton (loc. cit.) and Hammond et al (loc. cit.) was indeed an important landmark in the studies of bovine eimeriosis, globidiosis, and besnoitiosis. It brought forward convincing evidence that the intestinal parasite of Smith (1893), identified as E. bovis, and the cutaneous organism originally known as Besnoitia besnoiti are not identical as suggested by Nöller (1920) who referred to them as Globidium besnoiti. The identity of the parasites described as Gastrocystis smithi Railliet 1919, Globidium smithi Pillers 1928, and Globidium fusiformis Hassan 1935, still needs to be determined. It is possible that the schizonts seen in the former two parasites are those of E. bovis (Hammond et al, 1946).

Hammond et al (loc. cit.), who considered the life-cycle of E. bovis, raised the question whether this parasite is a true Eimeria sp. They pointed out that the asexual stage of the life history of E. bovis is different from the classical description of eimerian coccidia in parasitizing cells of tissue other than epithelial, in its location in the centre of a villus, in the large number of merozoites produced by a single schizont, and in the occurrence of a single generation of schizonts. (It is not clear why the host cell specificity should be used as a criterium for the classification of coccidia. A characteristic feature of Eimeria necatrix Johnson 1930, is the development of a very large generation of schizonts in the sub-epithelium of the intestine of fowls). In an attempt to identify the generic status of E. bovis correctly, they compared the life-cycle of this parasite with that of other protozoa which also show large cysts in the course of their development. They stated that a "series of investigations by different workers had brought

to light a pattern of development involving Globidium (the cystic stage of the parasite) and ending with Eimeria - type oocysts in the camel (Henry and Masson, 1932; Enigk, 1934; Naville, 1936), armadillo (Reichenow and Carini, 1937), and horse (Reichenow, 1940, Hemmert and Halswick, 1943). In these cases the Globidium stage is represented by the macrogametocyte and microgametocyte, and perhaps also the schizout, although this stage was not clearly worked out. The oocysts are usually large (about 75 microns long), and have a thick outer membrane around the oocyst proper. Henry and Masson (1932) suggested that a certain group of species of Globidium, including G. leuckarti in the horse, G. faurei (= G. gilruthi) in the intestine of sheep, G. cameli, in the camel, and G. besnoiti in the small intestine and skin of cattle, be defined as coccidia whose endogenous reproductive stages reach a large size, and large oocysts with thick walls. The group was to be placed in coccidia near or in the genus Eimeria. (It has already been pointed out above that there is not sufficient reason for grouping together the skin and intestinal forms of the so-called Globidium of cattle). Hammond et al. (loc.cit.) came to the conclusion that although it includes a Globidium stage, it does not fit into the pattern of the of the forms found in the camel, horse and armadillo. They arrived at the final conclusion "that the question of the systematic position of E. bovis can be better taken up when the life history of other species of coccidia in cattle, sheep and other hosts have been worked out in detail".

Reichenow (1953) adopted a less critical attitude towards the classification of the genus Eimeria and closely allied parasites. He concluded that the available information on the life-cycle of several of the Globidium species was adequate to incorporate them as a subgenus of the Eimeria. The subgenus Globidium Flesch 1883, he defined to incorporate eimeria-like parasites situated in the sub-epithelial tissues of the intestine. Their developmental stages, especially the schizonts and microgametocytes, attain an exceptionally large size

thereby causing a marked hypertrophy of the host cell. The oocysts are extremely large, and are enveloped by two capsules of which the external one is thick, of granular structure and with a wrinkled surface. Reichenow (loc.cit.) was aware that this definition does not embrace the features of all the species that he intended to include in the subgenus Globidium, and, therefore, stressed that there is no sharp demarcation between the Globidium and Eimeria s. str. In the case of E. bovis agamogony is typically that of a Globidium, while gamogony conforms with that of the Eimeria s. str. In his classification he referred to this parasite as Eimeria (Globidium) bovis (Züblin 1908) (Syn. Eimeria smithi Yakimoff and Galouzo 1927).

It has already been mentioned in this discussion that a great deal of confusion exists in the nomenclature of the parasite, causing cutaneous and subcutaneous lesions in cattle discovered by Besnoit and Robin (1912). Both investigators realized that it was a new parasite, and suggested that it may be a Sarcocystis sp. It was named Sarcocystis besnoiti by Marotel (1912). Since then the generic and specific nomenclatures of S. besnoiti have undergone several changes. Jellison (1956) who gave a comprehensive account on the nomenclature brought forward evidence that Henry (1913) used the generic name Besnoitia, used the combination Besnoitia besnoiti, and credited the authorship of this species to Marotel. Jellison (loc. cit.) pointed out that although Henry (1913) believed that Brumpt (1913) had established the genus Besnoitia in the second edition of his Précis de Parasitologie, in preparation, but scrutiny of this publication subsequently revealed that Brumpt had not proposed the generic name. On the contrary, Brumpt referred to the bovine cutaneous parasite as Gastrocystis Robini de la vache (Besnoit and Robin) on page 105, and as Gastrocystis besnoiti (Marotel) on page 113.

Franco and Borges (1916) formally proposed the generic name Besnoitia as they believed that the way of presentation by Henry (1913) did not comply with the nomenclatorial rules. In the Zoological Record of 1916, and in the Nomenclator Zoologicus by Neave (1939) Franco

and Borges (1916) were credited with the authorship of the generic name Besnoitia.

It has been mentioned before that Nöller (1920) revised the generic nomenclature of the genera Globidium, Ileocystis, Lymphocystis, Haplogastrocystis and Besnoitia, that he retained the genus Globidium, that he rejected the remaining generic names, that he concluded, without justification, that the cyst-like structures found in the intestine of cattle are identical with the cutaneous forms, and he included them under the name Globidium besnoiti Marotel 1912. In subsequent publications it will be seen that the name Globidium besnoiti was used by Wenyon (1926) Doflein and Reichenow (1929), Henry and Masson (1932) and Neveu-Lemaire (1943). Brumpt (1927, 1949) also followed Nöller (1920) in the change of the generic assignment but erred again in crediting the authorship to Besnoit and Robin instead of to Marotel, for he listed Globidium besnoiti de la vache (Besnoit and Robin).

Babudieri (1932), who revised the classification of the Sarcosporidia, came to the conclusion that the cutaneous parasite of cattle is a distinct species, and not related to the intestinal parasite. He transferred the cutaneous parasite to the genus Besnoitia, and listed it as B. besnoiti (Marotel 1912). In the revision of the classification of the Globidium spp. Reichenow (1953) came to the same conclusion. He referred to the cutaneous parasite as B. besnoiti (Marotel, 1912), and to the intestinal parasite as Eimeria (Globidium) bovis (Züblin 1908).

Up to this stage no studies had been conducted on the life-cycle of the bovine and equine Besnoitia spp. The mode of reproduction became evident when Frenkel (1953 a) described Besnoitia jellisoni in a white-footed mouse (Peromyscus maniculatus) (Wagner) Gloger. He established and maintained this parasite in laboratory animals. Microscopic observations revealed that this protozoon multiplies by binary fission, and that it bears a morphological resemblance to Toxoplasma gondii which also reproduces in the same fashion. Pöls (1954 a, b) transmitted B. besnoiti to rabbits, and maintained it successfully by serial passage in this animal. Studies

showed that the bovine parasite also reproduces by simple binary fission. These observations disproved the assumption of Nöller (1920) that the Besnoitia sp. multiplies by schizogony as had been determined for the intestinal parasite.

From this historical review it becomes apparent that no less than three generic names, namely Gastrocystis, Globidium and Eimeria, had been assigned to the intestinal protozoon Eimeria (Globidium) bovis, and that four generic names, namely Sarcocystis, Besnoitia, Gastrocystis and Globidium had been allotted to the cutaneous protozoon B. besnoiti. Jellison (1956), who scrutinized the nomenclature of the genus Besnoitia and of the species B. besnoiti and B. jellisoni, stated "that he offers no opinion on whether or not these two species deserve the distinction of a genus separate from Gastrocystis, Globidium or Sarcocystis". Comparison between the habitat, morphology, life-cycle and host-relationship of the Gastrocystis spp., Globidium spp., Sarcocystis spp. and Besnoitia spp. leaves no doubt that Besnoitia is a distinct genus, and that the two species B. besnoitia and B. jellisoni are according to available evidence stenoxenous parasites, a feature not possessed by members of the remaining three genera.

In order to obtain clarity about the identity of the two parasites of the horse, included in the genus Globidium, it will be necessary to review the relevant literature. Flesch (1883) found a parasite in cysts 300 by 170 microns in size in the villi of the small intestine of a horse in Germany. He named it Globidium leuckarti. This organism was again encountered by Hobmaier (1922) and Kupke (1923) in Europe. The latter investigator found in addition to the cysts harbouring merozoites, large unripe oocysts enveloped in a thick capsule in the villi of the small intestine. According to Neveu-Lemaire (1943) Henry and Masson (1922) found cysts, varying from 230 to 400 microns in diameter, which harboured numerous organisms in the cutis and subcutis of a horse in the vicinity of Reims in France. Since the spores within the cutaneous cysts showed some resemblance to the

intestinal parasite described by Flesch (1883) he identified the cutaneous protozoon as *Gl. leuckarti*. (It is obvious that Henry and Masson (loc. cit.) adopted the same scheme as Nöller (1920). Bennett (1927) described several cases of cutaneous sarcosporidiosis in horses from the Sudan. In a subsequent publication (Bennett, 1933) he stated that Wenyon had identified the causal agent as a Globidium sp.

Consideration of this account makes it apparent that two distinct types of globidiosis, namely the intestinal and the cutaneous forms occurred in horses, and that according to Henry and Masson (1922) Gl. leuckarti was responsible for both diseases. Babudieri (1932), who critically reviewed the classification of the class Sarcosporidia Bütschli 1882, concludes that Gl. leuckarti, originally described as the causal agent of intestinal globidiosis, is morphologically distinct from the parasite responsible for equine sclerodermatitis. Histological examination revealed that the equine cutaneous parasite, with its cystic membrane, is morphologically indistinguishable from B. besnoiti. He considered that the cutaneous parasite described by Bennett (1927) is specific for the horse, proposed that it be included in the genus Besnoitia, and listed it as Besnoitia bennetti Babudieri 1931. Bennett (1933), who described the presence of free spores in the areolar tissue of a horse, and the development of cysts harbouring the causal agent, comes to the conclusion that "in spite of the increase in the number of parasites, there is no suggestive arrangement of spores, or any other appearance to indicate that they have undergone but the simplest form of division". From these observations he suggests "that the organism now known as Globidium besnoiti, including in this species the parasite in the Sudan, is probably not a globidium or alternatively that the definition of the genus Globidium needs redrafting. The decision must be left to those better qualified to make it, but it seems that the species Sarcocystis besnoiti might still be considered good". Enigk (1934) who carefully studied the life-cycle of *G. cameli* and that of Gl. leuckarti, came to the conclusion that the equine cutaneous parasite, described by Bennett (1933) is not a Globidium sp.

The proposal of Babudieri (loc. cit.) was not generally accepted. Bennett (1937) still referred to the cutaneous parasite as a Globidium sp. Neveu-Lemaire (1943) continued to consider that the intestinal and cutaneous parasites are identical, listed it as Gl. leuckarti, and retained it in the sub-family Eimerinae Henry and Masson 1932. Although Reichenow (1953) did not accept Babudieri's classification of the class Sarcosporidia, he agreed that the correct name for the cutaneous parasite is B. bennetti.

Investigations conducted on the life-cycle of the intestinal parasite (Gl. leuckarti) by Reichenow (1940) revealed the presence of immature oocysts in the faeces of a horse and a donkey. After 14 days the oocysts showed four sporoblasts, and seven days later the ensuing sporocysts each harboured two sporozoites. These observations caused this investigator to include the parasite in the genus Eimeria. Studies by Hemmert-Halswick (1943) in Germany and Hiregaular (1956) in Pakistan confirmed the previously observed eimerian features of the intestinal parasite (Gl. leuckarti). Reichenow (1953) listed this organism as Eimeria (Globidium) leuckarti (Flesch 1883).

From this historical review it becomes apparent that the two generic names Globidium and Eimeria had been assigned to the equine intestinal parasite, that the former genus received a sub-generic status, and that it was thereupon included in the genus Eimeria. Three generic names Globidium, Sarcocystis and Besnoitia had been allotted to the cutaneous parasite. The former two names were rejected while Besnoitia was accepted as the correct generic name. Detailed studies on the life-cycle of the cutaneous protozoon have not yet been carried out but there is every reason to believe that the conclusion of Bennett (1933) that parasite divides by binary fission will be confirmed.

Scrutiny of the literature on the abomasal and intestinal parasites of sheep and goats has shown that confusion still exists about the identity of Gl. gilruthi Chatton 1910. Maske (1893) found cysts up to 1000 microns

in diameter, harbouring numerous spores, in the abomasal mucous membrane of sheep in Germany. He regarded this parasite to be related to the gregarines. Moussu and Marotel (1902), who encountered this abomasal parasite in sheep from France, believed that it represented a developmental phase of Eimeria faurei Moussu and Marotel 1901. Gilruth (1910) recorded the occurrence of abomasal cysts harbouring blastophores and spores in Australian sheep. Chatton (1910) encountered the same parasite in the abomasum and small intestine of sheep and goats from Australia. He first named the parasite Gastrocystis gilruthi in 1910, and during the same year included it in the genus Globidium. Neveu-Lemaire (1943) listed the protozoon as Globidium faurei (Moussu and Marotel 1902), and incorporated it in the sub-family Eimerinae. Reichenow (1953) on the other hand provisionally included this organism in the genus Eimeria on the assumption that the abomasal and intestinal cysts represent the schizogonous phase, and that the gamogonic phase, which has not yet been determined experimentally, probably develops in the distal portion of the small intestine. He suggested that Eimeria intricata Spiegl 1925, which possesses a relatively large thick-walled oocyst probably represents the sporogonist phase in the life-cycle of Gl. gilruthi. He tentatively transferred this protozoon to the genus Eimeria, included it in the subgenus Globidium, and listed it as Eimeria (Globidium) gilruthi. (Chatton 1910) (syn: Gastrocystis gilruthi Chatton 1910).

Investigations on this group of parasites continued. In a dromedary (Camelus dromedarius Lin.) submitted at the Alfort Veterinary College in France, Henry and Masson (1932) discovered at autopsy mature macrogametocytes, and a very large thick-walled immature oocyst in the mucosa of the small intestine. They named the parasite Gl. cameli. Enigk (1934), who worked in Germany, found the same parasite not only in the jejunum and ileum but also in the caecum of a camel (Camelus bactrianus Lin.) which originally came from Uralsk. He described immature and mature schizonts, microgametocytes and large unripe oocysts. The mature forms of the former two stages measured 200 by 150 microns. Reichenow (1953) cast doubt

on Enigk's interpretation of the agamonic phase in which minute merozoites 2.0 microns in length appeared. He suggested that the alleged schizonts actually represented developing microgametocytes. Consideration of the nature of the gamogonic and sporogonic stages caused Reichenow (loc. cit.) to transfer Gl. cameli to the genus Eimeria, to include it in the subgenus Globidium, and to list it as Eimeria (Globidium) cameli Henry and Masson 1932.

In Brazil Globidium-like parasites were found in two armadillo species. Gl. travassosi Da Cunha and Muniz 1928, was found in the subepithelial tissues of the small intestine of the six-banded armadillo (Dasyus sexsinctus Lin. = Euphaetus sexsinctus (Lin)). The developmental stages appeared in markedly hypertrophied host cells. A detailed description of the gamogonic stages, and of the immature and mature oocysts was given by Reichenow and Carini (1937). They stated that the agamogonic phase was represented by a single schizont 100 by 84 microns in size containing numerous merozoites. They expressed the opinion that the figures quoted did not represent the actual dimensions of the schizont, as the single section available, had been cut near the margin, and that the maximum size was in all probability much greater. Reichenow and Carini (loc. cit.) suggested that this parasite may actually be identical with Gl. tatusi Da Cunha and Torres 1926, described from the nine-banded armadillo (Dasyus novemsinctus (Lin.) = Tatus novemsinctus Lin.) The mature schizonts of this parasite, the only stage seen, can attain the tremendous size of 2100 by 500 microns. Should this prove to be correct then Gl. travassosi becomes synonymous for Gl. tatusi. Reichenow and Carini (loc. cit.) transferred Gl. travassosi tentatively to the genus Eimeria, included it in the subgenus Globidium, and listed it as Eimeria (Globidium) travassosi Da Cunha and Muniz 1928.

For the sake of completeness the remaining protozoa included in the genus Globidium will be considered in this review. They comprise parasites of Australian marsupials and European snakes. It will be noticed that only cysts containing either blastophores and/or spores which may represent schizonts of an Eimeria were described.

Before discussing the intestinal parasites of the marsupials it must be mentioned that Reichenow (1953) stated that the knowledge about their developmental cycles is incomplete, and that their systematic status still needs to be determined.

Blanchard (1885) found sarcosporidia-like cysts, varying from 710 to 1230 microns in length and 510 to 950 microns in width, and harbouring kidney-shaped spores 9.8 to 12.0 microns in length in the connective tissue of the small intestine of a kangaroo (Macropus penicillata (Gray) = Petrogale penicillata Gray). According to Wenyon (1926) he named the parasite Sarcocystis mucosae, while Reichenow (1953) stated that he named it Balbiania mucosa. Coutelen (1933), who re-examined the intestinal globules, found that the cysts were chambered, and, therefore concluded that the parasite is a Sarcocystis sp.

Gilruth and Bull (1912) recognised two types of cysts in a kangaroo which had died from haemorrhagic enteritis. The cysts were situated in the subepithelial tissues of the markedly inflamed small intestine. The large cysts, with a diameter varying from 70 to 334 microns occurred in groups situated in the deep layers of the mucous membrane. Single cysts, with a distinct membrane, appeared within hypertrophied cells. Free spores were not seen. They named the parasite Ileocystis macropodis. Gilruth and Bull (1912) created a new genus Haplogastrocystis and changed the name I. macropodis to Haplogastrocystis macropodis. Nöller (1920), who revised the generic nomenclature regarded, without justification, that Balbiania mucosa is identical with H. macropodis, included them in the genus Globidium, and used the combination Gl. mucosa Blanchard 1885? as possibly the correct name.

The smaller cysts were demonstrated in the connective tissue of the mucosa in great numbers. When mature they had a diameter of 8.4 microns, and were filled with spores 5.5 microns long and from 2.0 to 2.5 microns broad. The cystic wall was extremely thin, and

/39.....

contained a compressed nucleus. It appeared that the host cell was a mononuclear leucocyte. This parasite was named Lymphocystis macropodis Gilruth and Bull 1912. This protozoon was not included in the genus Globidium by Nöller (1920).

In two wallabies (Petrogale sp.) which had died from enteritis, and in an apparently healthy one Gilruth and Bull (1912) found cysts, arranged in groups, in the submucosa of the small intestine. The cysts had a multilocular structure, the wall of which consisted of two membranes. They contained numerous blastophores which gave rise to spores. Chatton (1912) suggested that this parasite be included in the genus Haplogastrocystis. Nöller (1920) listed it as a Globidium spec. Gilruth and Bull 1912.

Wenyon and Scott (1925) and Triffit (1926) described parasites in the black wallaby (Macropus ualabatus (Lesson and Gurnior) = Macropus bennetti Lesson and Gurnior) which resembled I. macropodis and L. macropodis. They were, however, unable to determine whether these parasites were related. In addition they found that the muscle fibres of the intestine contained elongate vacuolic spaces filled with spores, which appeared very similar to those of the Lymphocystis type. Whether this, again, is a species of Sarcocystis or is another stage of Lymphocystis remained obscure. Wenyon and Scott (loc. cit.) concluded that if three parasites were represented, then in the portion of the intestine, which they examined, there occurred four distinct species as an Eimeria sp. was also present in the epithelium. In conclusion they suggested that the parasite S. mucosae Blanchard 1885 may be identical with I. macropodis.

In a hairy nosed wombat (Lasiorhinus latrifrons (Owen) = Phascologomys latrifrons Owen), that died at Melbourne, and which showed a chronic catarrhal papillomatose enteritis of the small intestine, Gilruth and Bull (1912) found a parasite which they named Ilaecystis wombati. This protozoon occurred within round or oval thick walled cysts varying from 92 to 114 microns in size. The cysts were situated within the proliferating connective tissue of the hypertrophied villi and also within the glands of

Lieberkühn. In structure it resembled Gl. gilruthi. Chatton (1912) suggested that this parasite should be included in the genus Haplogastrocystis while Nöller (1920) transferred it to the genus Globidium, and listed it as Gl. wombati Gilruth and Bull 1912.

The last Globidium species to be considered is Gl. navillei Harant and Gazal 1934, which has been recorded from two snakes Tropidonotus natrix Lin. and T. viprinus Lin. Schizonts up to 500 microns in size appeared in the markedly hypertrophied host cells in the subepithelial tissue of the intestinal mucosa. Gamogonic stages and oocysts were not observed. The suggestion of Naville (1931) that these large schizonts represented the agamogonic phase of Eimeria tropidonoti Guyenot, Naville and Ponce 1922, was not accepted by Harant and Gazal (loc.cit.). In order to avoid an unnecessary nomenclatorial confusion Reichenow listed the protozoon as Gl. navillei Harant and Gazal 1934.

The last genus that needs attention is Fibrocystis Hadwen 1922. The first species, F. tarandi Hadwen 1922, to be described was found in fibrous tissue, especially that covering the tendons and the periosteum of the reindeer (Rangifer tarandi Lin.). The cysts have a diameter of 100 to 450 microns, while the cystic wall consist of three layers enclosing numerous spores. This parasite causes "corn meal disease", and when in the periosteum the cysts cause atrophy of the bones which appear pitted. Wenyon (1926) suggested that it may be related to the Sarcosporidia, while Babudieri (1932) was of opinion that it may be allied to the genus Besnoitia. The second species, F. darlingi (Brumpt 1915), of the opossum (Didelphis sp.) was originally included in the genus Sarcocystis by Brumpt (1915). It occurs in non-chambered oval cysts, varying from 1500 to 2000 microns in size, which are situated in the connective tissue of parenchymatous organs. The structure of the cyst, and the habitat of the parasite, which differs from those of a typical Sarcocystis sp. caused Babudieri (1932) to transfer this parasite to the genus Fibrocystis.

/41.....

Having surveyed in detail the complicated history of the nomenclature of certain Protozoa included in the general Sarcocystis, Eimeria, Globidium, Balbiania, Gastrocystis, Ileocystis, Lymphocystis, Haplogastrocystis, Besnoitia and Fibrocystis, and having indicated the criteria employed in their classification, the views expressed by several protozoologists about their status in the higher taxonomic categories will be submitted in chronological order.

Nöller (1920) stated that before the complete developmental cycle of a representative of a genus is not known in detail, it is impossible to arrange it in the classificatory system of the Protozoa. He reviewed the relevant literature up to the year 1918, and stressed that the taxonomic studies by several investigators had not brought forward a clearer concept about their identity. He regarded the assignment of various generic names to certain intestinal and cutaneous parasites as futile. He recommended that for the sake of simplicity the parasites be included in the genus Globidium Flesch 1883, and that the other generic names be regarded as synonyms. In his revised generic nomenclature he listed the following species together with their synonyms as:-

Globidium leuckarti Flesch 1883. (Equus caballus Lin.)

Globidium gilruthi Chatton 1910.

(Ovis aries Lin. and Capra hircus Lin.)

Synonym: Gastrocystis gilruthi Chatton 1910.

Globidium besnoiti Marotel 1912. (Bos taurus Lin.)

Synonyms: Gastrocystis besnoiti Marotel 1912,  
Besnoitia nov. gen. Franco & Borges 1916.

Globidium mucosa Blanchard 1885? (Macropus penicillata (Gray))

Synonyms: Ileocystis macropodis Gilruth and Bull 1912,  
Haplogastrocystis nov. gen. Chatton 1912.

Globidium sp. Gilruth and Bull 1912. (Petrogale sp.).

Synonym: ? Sarcocystis macropodis Gilruth and Bull 1912

Globidium wombati Gilruth and Bull 1912.

(Lasiorhinus latrifrons (Owen))

Synonyms: Ileocystis wombati Gilruth and Bull 1912.  
Haplogastrocystis nov. gen. Chatton 1912.

Although the species Lymphocystis macropodis Gilruth and Bull 1912, was discussed by Nöller (1920) under Gl. mucosa, it was not included in the genus Globidium. It will also be noticed that the synonyms Sarcocystis besnoiti Marotel 1912, and Gastrocystis robini Brumpt 1913, are not listed as synonyms of Gl. besnoiti.

Wenyon (1926) referred to the abovementioned organisms as parasites of undetermined position. He stated "that there are a number of organisms which are usually grouped with the Cnidosporidia, though they do not show any of their characters. It is doubtful if some are Protozoa at all. The chief of these are the Sarcosporidia, which are parasitic in the muscle fibres of vertebrates, and have the form of elongate chambered bodies filled with sickle-shaped spores; the closely related Globidium (cysts of Gilruth), which give rise to nodules in the mucosa of the stomach and intestine of ruminants and others; the Haplosporidia, which occur chiefly in invertebrates in the form of uninucleate or multinucleate cytoplasmic bodies and resistant spores; and the Serumsporidia, which are found in the body-cavity fluids of aquatic crustacea and larvae as small round cells which multiply by binary fission or schizogony. The Rhinosporidia, which produce nasal polypi, have been usually classed with the Haplosporidia, but Ashworth (1923) has shown conclusively that they are fungi, and not Protozoa, and this appears to be true of Globidium and possibly the Sarcosporidia".

Wenyon (1926) considered that parasites included in the genus Globidium Flesch 1884, (date should be 1883) "are probably related to the Sarcosporidia, have the form of spherical cysts up to 5 millimetres in diameter embedded in the mucosa of the alimentary canal or skin of mammals. Each is enclosed by a membranous capsule, and when fully grown consists of spores which resemble those of the Sarcosporidia".

Under the Sarcosporidia Bütschli 1882, Wenyon (1926) listed the following parasites:-

/43.....

Globidium leuckarti Flesch 1884. (Equus caballus Lin.)

Globidium gilruthi Chatton 1910.

(Ovis aries Lin., and Capra hircus Lin.)

Synonyms: Gastrocystis gilruthi Chatton 1910;  
Eimeria faurei Moussu and Marotel 1902.

Globidium besnoiti Marotel 1912. (Bos taurus Lin.).

Synonyms: Sarcocystis besnoiti Marotel 1912.  
Besnoitia nov. gen. Franco and Borges  
1916.

Globidium tatusi Da Cunha and Torres 1924.

(Dasypus novemlineatus (Lin.))

Sarcocystis mucosae Blanchard 1885. (Macropus sp.).

Synonym: ? Ileocystis macropodis Gilruth and  
Bull 1912.

Sarcocystis macropodis Gilruth and Bull 1912.

(Petrogale sp.).

Ileocystis macropodis Gilruth and Bull 1912.

Macropus penicillata (Gray) and  
M. ualabatus Lesson and Gurnior).

Synonym: ? Lymphocystis macropodis Gilruth and  
Bull 1912.

Ileocystis wombati Gilruth and Bull 1912.

(Lasiornis latifrons (Owen)).

Lymphocystis macropodis Gilruth and Bull 1912.

Macropus penicillata (Gray), and  
M. ualabatus (Lesson and Gurnior))

Fibrocystis tarandi Hadwen 1922, (Rangifer tarandus Lin.)

Reichenow (1929) came to the conclusion that there is no convincing evidence that the parasites under discussion are Protozoa, and that only careful studies on their life-cycles would reveal their true identity. He stated that the views expressed by Chatton (1910) and Nöller (1920) on the gregarine-like development appeared to be more acceptable than those of other investigators who had assumed that these parasites belong to the Sarcosporidia. In this review (Reichenow loc. cit.) used the name Gl. besnoiti for both the intestinal and cutaneous parasites of cattle and Gl. leuckarti for both the intestinal and cutaneous parasites of the horse.

/43.....

He gave a brief account of the intestinal parasites of the Australian marsupials, and refrained from mentioning their names. He listed the following species:-

Globidium leuckarti Flesch 1883. (Equus caballus Lin.)

Globidium besnoiti Marotel 1912, (Bos taurus Lin.)

Globidium gilruthi Chatton 1910.

(Ovis aries Lin. and Capra hircus Lin.)

Globidium tatusi Da Cunha and Torres 1926.

(Dasypus novemsinctus (Lin.)).

Babudieri (1932) carefully reviewed the literature of the Sarcosporidia, and that of the parasites under discussion. He came to the conclusion that the latter possessed characteristics which permitted their inclusion in the sub-class Sarcosporidia. In doing so he created two new orders and three new families. The revised classification is as follows:

Class: Sporozoa Leuckart 1879.

Sub-Class: Sarcosporidia Balbiani 1885.

This sub-class comprises Sporozoa situated in striated and non-striated muscles and connective tissue of mammals, birds and reptiles. The parasites appear as banana-shaped or fusiform structures within cysts formed by either the host, the parasite or by the combined action of the host and parasite. The cystic cavity may or may not be chambered. The spores harbour a nucleus, usually granular and with a distinct nucleolus. In the middle of the spore metachromatic and siderophilic granules are visible.

Order: Sarcosporidia Bütschli 1882.

The order includes banana-shaped spores situated within cysts, which are either totally or partially of parasitic origin. Spores are provided with metachromatic granules, and secrete a toxin known as sarcocystin. They are derived from proliferating pansporoblasts and sporoblasts situated along the margin of the cyst.

Family: Sarcocystidae Babudieri 1932.

The family is represented by a single genus Sarcocystis which comprises Sarcosporidia situated exclusively in striated muscles. Cysts are elongated and sub-divided by septa, and seldom cause an inflammatory

reaction.

Genus: Sarcocystis Ray Lankester 1882.  
S. miescheriana Kuhn 1865. (Sus scrofa Lin.)

In addition 21 Sarcocystis spp. occurring in either mammals, birds or reptiles are listed.

Family: Fibrocystidae Babudieri 1932.

The family comprises Sarcosporidia of the connective tissue and non-striated muscle. Cysts are large and round, not subdivided by septa, and are surrounded by a thick fibrous capsule. The genus Fibrocystis is situated in the deep connective tissue and periosteum and connective tissue of parenchymatous organs, and rarely affects the host. The genus Besnoitia is situated mainly subcutaneously and is highly pathogenic.

Genus: Fibrocystis Hadwen 1922.  
F. tarandi Hadwen 1922.  
(Rangifer tarandus Lin.).  
F. darlingi Brumpt 1915 (Didelphis sp.).  
Synonym: Sarcocystis darlingi Brumpt 1915.  
Besnoitia Franco and Borges 1916.  
B. besnoiti Marotel 1912. (Bos taurus Lin.)  
B. bennetti Babudieri 1931.  
(Equus caballus Lin.).

Order: Globidia Babudieri 1932.

The order comprises parasites with a few siderophilic granules in the spores which do not secrete a toxin. Cysts are situated exclusively in the submucosa of the alimentary tract. The host cell is a hypertrophied somatic cell, the nucleus of which has a marginal situation. The spores are derived from mulberry-like masses, the so-called blastophores.

Family: Globididae Babudieri 1932.

The family includes two genera. The genus Globidium is characterised by multiple blastophores, and the genus Ileocystis by a single blastophore within the cyst.

/46.....

Genus: Globidium Flesch 1883.  
G. leuckarti Flesch 1883. (Equus caballus Lin.).  
G. gilruthi Chatton 1910. (Ovis aries Lin.).  
G. besnoiti Marotel 1912. (Bos taurus Lin.).  
G. tatusi Da Cunha and Torres 1926.  
 (Dasypus novemcinctus (Lin.)).  
G. macropodis Gilruth and Bull 1912.  
 (Macropus penicillata (Gray),  
M. bennetti Owen, Petrogale sp.)

Genus: Ileocystis Gilruth and Bull 1912.  
I. macropodis Gilruth and Bull 1912.  
 (Macropus penicillata (Gray)).

Reichenow (1953) briefly reviewed the classification of Babudieri (1932), and stressed that the taxonomy of this group of organisms is unsatisfactory as in many cases the available knowledge about their life-cycles is incomplete. He pointed out that members of the order Sarcosporidia are usually included in the single genus Sarcocystis but that the incorporation of the genera Fibrocystis and Besnoitia, occurring in unilocular cysts, into this order is very problematic. However, he agrees that members of these genera are distinct from the Globidium spp. of the alimentary tract as studies on the life-cycle of the latter, in most cases, had shown that they belong to the coccidia. It will be seen from Reichenow's revised classification that he retained the intestinal parasites of the marsupials and snakes in the subgenus Globidium but that he refrained from listing their names in the genus Eimeria as there is no evidence that gamogony and sporogony occurs in their developmental cycle.

Reichenow's revised classification of the genus Globidium is as follows:-

Class: Sporozoa Leuckart.  
 Sub-Class: Telesporidia Schaudinn.  
 Order: Coccidia Leuckart.  
 Sub-Order: Eimeridea Léger.  
 Family: Eimeridae Léger emend.  
 Genus: Eimeria Schneider 1875.  
 Sub-genus: Globidium Flesch 1883.  
 Synonym: Gastrocystis Chatton 1910.

Eimeria (Globidium) leuckarti Flesch 1883.

(Equus caballus Lin.).

Eimeria (Globidium) cameli Henry and Masson 1932.

(Camelus bactrianus Lin. and C. dromedarius Lin.)

Eimeria (Globidium) gilruthi (Chatton 1910).

(Ovis aries Lin. and Capra hircus Lin.).

Synonym: Gastrocystis gilruthi Chatton 1910.

Eimeria (Globidium) travassosi (Da Cunha and Muniz 1928).

(Dasyus sexsinctus (Lin.)).

? Eimeria (Globidium) tatusi Da Cunha and Torres 1926.

(Dasyus novemsinctus (Lin.)).

Synonym: ? Eimeria (Globidium) travassosi  
da Cunha and Muniz 1928.

Eimeria (Globidium) bovis (Züblin 1908) (Bos taurus Lin.)

Synonym: Eimeria smithi Yakimoff and  
Galouzo 1928.

Consideration of the investigations conducted during the last five decades makes it apparent that sufficient evidence has been brought forward that B. besnoiti is neither a coccidium nor a sarcosporidium but that the determination of its identity was largely dependent upon studies of the life-cycle. Bennett (1933) concluded from his histological studies that B. besnoiti of the horse (now known as B. bennetti) in all probability multiplied by the simplest form of division, namely binary fission. A profitable method of approach for these studies was offered by Cuillé, Chelle and Berulaeu (1936) and Barrairon (1938), who showed that bovine besnoitiosis can be reproduced artificially in cattle by administering infective blood by either the subcutaneous or intravenous route. This was indeed another important landmark in the investigations on besnoitiosis, but it was left to Frenkel (1953 a, 1956) to finally disclose the vertebrate life-cycle and immunogenic properties of the genus Besnoitia. His studies on B. jellisoni not only revealed that this protozoon reproduced by repeated binary fission but that it is morphologically related to, though immunologically

distinct from the heteroxenous parasite T. gondii. Frenkel's observations were confirmed by Pols (1954 a, b) who showed that B. besnoiti is endowed with similar properties as B. jellisoni, and that the derivation of the cystic wall, which had given rise to a great deal of speculation, is of somatic origin.

Having surveyed in detail the complicated history of the nomenclature of the Besnoitia spp. and Fibrocystis sp., and having brought forward evidence that members of both genera are related to T. gondii, it will be necessary to give a brief review of the nomenclatorial history of the toxoplasms as in recent years the classification has been modified.

The genus Toxoplasma is characterised by oval, crescentic or reniform parasites, 2.5 to 6.5 microns in length and 1.5 to 3.5 microns in breadth, possessing a single nucleus which is mostly central but sometimes it is nearer one end than the other. The ends of the body are usually pointed, one end usually being blunter and broader than the other. The parasites multiply by longitudinal binary fission, and gradually fill and distend the host cells, the reticulo-endothelial cells, mononuclear leucocytes, and more rarely polymorphonuclears. These aggregations are referred to as pseudocysts, are usually rounded, vary from 5 to 50 microns in diameter, and harbour up to one hundred or more parasites. The natural mode of transmission is obscure. It has been suggested that the infection is acquired by ingestion of food contaminated with infected faeces or some arthropod vector. Toxoplasms may be pathogenic to both naturally and experimentally infected animals and the ensuing disease may have a fatal termination.

Species names have been assigned to a large number of toxoplasms recorded from mammals and birds. However, investigations by Carini (1909) and Manwell and Drobeck (1951) have shown that the parasites described from different mammalian hosts are not only morphologically but also immunologically identical. The fact that T. gondii will infect rabbits, guinea-pigs, dogs, mice, moles, pigeons and Java sparrows, in all of

which naturally occurring toxoplasma infections have been found, raises the question of the identity of various species (Wenyon, 1926). It thus appeared that convincing evidence had been brought forward that the genus Toxoplasma is represented by a single species, T. gondii.

Garnham (1950) modified this conception after comparing the morphology of T. gondii with that of the toxoplasma-like organisms encountered in naturally infected birds. He brought forward evidence that the mammalian and avian parasites are distinct, and created the genus Atoxoplasma which comprises avian parasites from many parts of the world which are strictly host specific, non-pathogenic, possess a delicately granular cytoplasm not enclosed in a periplast, and a large diffuse nucleus with a tiny karyosome. Atoxoplasma avium (Marullaz 1913) was selected as the type species. Laird (1959), who established Atoxoplasma parasites in several species of birds belonging to the family Zosteropidae in the South Pacific region, came to the conclusion that they are not host specific and stated that Lainson (1958) had demonstrated that the Atoxoplasma may be pathogenic. He concluded that "the affinities of Atoxoplasma lies not with the toxoplasma but with the haemogregarines, and that failure to achieve transmission by subinoculation is not due to strict host specificity but to the fact that the method employed has simply not resulted in the transfer of stages able to establish infection in another vertebrate". He therefore, proposed to amend the diagnosis of Atoxoplasma as follows:-

"Benign to mildly pathogenic parasites, typically sausage-shaped with more or less equally rounded ends; occurring in avian monocytes and lymphocytes, often causing pronounced indentation of the nucleus of the latter; their weak staining reaction and the absence of a well-defined periplast rendering it difficult to differentiate their cytoplasm from that of the host cell; nucleus diffuse, granular and with a tiny karyosome; multiplication by binary fission, few (usually 2) division products per host cell; not transmissible by subinoculation".

Laird (1959) reviewed the literature on the Atoxoplasma spp. and Toxoplasma spp. of birds, and stated that according to the International Rules of Zoological Nomenclature Atoxoplasma paddae (Aragao 1911)

(syn. Haemogregarina paddae Aragao 1911) must, therefore, be regarded as the type species of the genus Atoxoplasma. Consideration of the investigations makes it apparent that the genus Atoxoplasma is distinct from the genus Toxoplasma and that the former need not be considered in the generic classification of the Toxoplasma, Besnoitia and Fibrocystis parasites.

The last question that arises out of this discussion is the status of the genera Toxoplasma, Besnoitia and Fibrocystis in the higher taxonomic categories. Wenyon (1926), Neveu-Lemaire (1943) and Reichenow (1953) concluded that neither the morphology nor the mode of reproduction of Toxoplasma present any characteristic features, and hence this genus cannot be placed in any natural scheme of classification of the Protozoa. Westphal (1953) expressed the opinion that the genus Toxoplasma may be related to the genus Leishmania Ross 1903, from which it can be differentiated by the absence of the blepharoplast. In support of this theory he drew attention to Trypanosoma equinum Voges 1901, which has lost its blepharoplast, and suggested that the same could have happened with the Toxoplasma.

In conclusion the writer wishes to state that he favours the conservative attitude adopted by (Wenyon 1926), Neveu - Lemaire (1943) and Reichenow (1953), and proposes that for the present the genera Besnoitia and Fibrocystis be included with the genus Toxoplasma in the group of "Parasites of Doubtful Nature".

The incomplete classification of these three genera is as follows:-

Genus: Toxoplasma Nicolle and Manceaux 1909.  
T. gondii Nicolle and Manceaux 1909.  
(Ctenodactylus gundi (Rothmann))

Synonyms: T. pyrogenes Castellani 1913. (Man).  
T. canis De Mello 1910. (Dog).  
T. cuniculi Splendore 1908. (Rabbit).  
T. ratti Sangiorgi 1914. (Rat).  
T. caviae Carni and Migliano 1916.  
(Guinea pig).  
T. sciuri Coles 1914. (Squirrel).

/51.....

T. talpae Prowazek 1910. (Mole).

Genus: Besnoitia Henry 1913.

B. besnoiti (Marotel 1913) (Cattle, sheep,  
goat and rabbits).

Synonyms: Sarcocystis besnoiti Marotel 1912.

Globidium besnoiti (Marotel 1912).

Gastrocystis besnoiti (Marotel 1912).

B. bennetti Babudieri 1913.

Synonym: Globidium besnoiti (Marotel 1912).

B. jellisoni Frenkel 1953.

Genus: Fibrocytis Hadwen 1922.

F. tarandi Hadwen 1922. (Reindeer).

### Transmission.

A. Natural Transmission:- The natural mode of transmission of B. besnoiti is obscure. Investigators in this field of research have advanced several theories which comprise (a) transmission by contact, (b) mechanical transmission and (c) biological transmission.

(a) Transmission by contact:- Bennett (1933) and Hofmeyr (1945) suggested that transmission follows contact between susceptible and affected animals. In an attempt to confirm this assumption affected cattle were allowed to cohabit with fully susceptible animals over a period of ten years at Onderstepoort. All animals had access to the same food manger and water trough. No transmission resulted. These results permit the conclusion that cohabitation is not followed by transmission.

(b) Mechanical transmission:- In order to obtain evidence whether this method of transfer is possible the writer employed rabbits showing a microscopic blood infection of B. besnoiti as reservoirs. Transmission could be obtained readily by introducing the tip of a seventeen gauge hypodermic needle into the ear vein of the donor, and immediately after its removal inserting it either into an ear vein or into the skin of several rabbits. This successful transmission indicates the possibility of a mechanical transmission by arthropods during

the period of a microscopic blood infection. It must, however be stressed that the period of an active infection is of short duration in cattle and that mechanical transmission would be a matter of chance. Even if one did accept that this method of transfer does occur, one is still faced with the question how the first cases are produced at the beginning of the bovine besnoitiosis season when the only known source of infection are the convalescent cyst carriers. Should such animals serve as reservoirs then one must expect that the vector must be endowed with powerful and deeply penetrating mouth parts capable of piercing the cyst wall. Comparison between the mouth parts of various blood sucking insects suggests that members of the family Tabanidae may fulfil these requirements. It thus appears that this avenue of approach may be fruitful for determining the mode of transmission.

(c) Biological transmission:- The seasonal incidence of bovine besnoitiosis caused Barrairon (1938) and Herin (1952) to suggest that this disease may be transmitted by an arthropod. They did, however, not state specifically whether transmission is effected mechanically or biologically. The requirements for the former method have already been discussed. Should the latter method be involved then the vector must also possess well developed mouth parts, and be capable of surviving the interepizootic period. This is not impossible if one considers the seasonal incidence of sweating sickness where Hyalomma truncatum Koch, with its strongly developed mouth parts is responsible for the transmission. Although there is no evidence that ticks are involved in the transmission it is nevertheless suggested that this avenue be explored.

#### B. Artificial transmission.

(a) Attempts to transmit the disease with cutaneous parasites:- Besnoit and Robin (1912) tried to transmit B. besnoiti to cattle by injecting cyst suspensions by either the intracutaneous or subcutaneous route but without success. The insertion of finely cut infected skin strips into the skin of susceptible animals did not result in transmission. Franco and Borges (1916) failed to infect rats and mice by administering cyst suspensions by either the oral or subcutaneous route.

What appears to have been a successful transfer was that recorded by Cuillé and Chelle (1937). They injected a cyst suspension intravenously into an ox which developed a bout of fever 15 days later. Unfortunately they omitted to examine skin sections subsequently, and hence it is impossible to state whether they had succeeded in transmitting the infection.

During 1953 the writer repeated this work using pieces of skin from heavily parasitized cattle. These specimens were suspended in physiological saline and finely mixed in a waring blender. Examination of the suspensions revealed the presence of numerous motile spores. The inoculum in the form of an opaque suspension was administered intravenously into two oxen and subcutaneously into two other recipients at the rate of 5.0 ml per 50 Kg. body weight. In another instance a thin sheet of connective tissue approximately 4 square cm. in size and heavily infected with Besnoitia cysts was implanted subcutaneously into the flank of an ox. The body temperatures of the five recipients were recorded twice daily over a period of two months. Visual inspection was continued for another two months. In not a single instance could any clinical evidence of besnoitiosis be detected. The writer erred in the same way as Cuillé, Chelle and Berlureau (1936) and omitted to examine skin sections for the presence of parasitic cysts, and hence it is unknown whether or not a subclinical infection had occurred.

(b) Attempts to transmit the disease with blood parasites:-

- (1) Infection of cattle:- Failure to reproduce clinical cases of bovine besnoitiosis by means of cyst suspensions suggested that the work Cuillé et al (1936, 1937) be repeated by using blood of actively infected cattle as inoculum. An opportunity arose for such tests during December 1953, and January and February 1954, when three head of naturally infected cattle showing the early stages of the disease became available. One litre of blood from each of these animals was transfused into three fully susceptible cattle Nos. 6793, 6839 and 5895. The two donors (Nos. 6840 and 6851) and the three recipients were observed, and blood and lymph node smears were examined daily until the

primary stage of the disease had passed. Skin biopsies from the dewlap and/or escutcheon were taken at four to five days intervals for a period of two months, and subsequently once a fortnight until a period of six months had lapsed. The skin specimens were sectioned and stained with Haemalum-eosin.

The results of these observations have been reported upon by Pols (1954 a) and are again detailed in Table B of appendix. It will be seen that the three recipients developed besnoitiosis as judged by eventually positive skin biopsies, even though the infection in one case (No. 6793) was clinically inapparent. In two cases no parasites could be detected in blood and lymphatic gland smears, while in the one (No. 6839) organisms were visible in the blood eleven days after artificial infection i.e. one day after the commencement of fever. Cysts were first observed in skin biopsies 23 to 34 days after infection and 13 to 28 days after the initial rise in temperature.

One further ox to ox transmission was undertaken using 250 ml. blood from an infected rabbit by the intravenous route. In the recipient (No. 5870) B. besnoiti appeared in the blood nine days later, simultaneously with the temperature elevation. No attempt was made to search for cutaneous cysts.

(2) Infection of rabbits, mice, rats and guinea pigs:-

Consideration of the course of the disease in artificially infected cattle makes it apparent that this species is not suited for large scale experiments, and that the maintenance of B. besnoiti in one or other species of laboratory animals would greatly facilitate investigations. (Pols, 1954 a).

With this object in view infected blood of a naturally infected ox No. 6840 was used as inoculum, and injected either intraperitoneally or subcutaneously into six mice (1.0 to 2.0 ml.), four rats and four guinea pigs (2.0 to 3.0 ml.). It was impossible to state whether or not they had developed an inapparent

form of the disease. On the other hand it was determined that rabbits are highly susceptible (Pols, 1954 a). Three rabbits (RH, RB and RT) were infected with blood using 3.0 ml. intravenously, 5.0 ml. intraperitoneally and 10.0 ml. subcutaneously, respectively from the same donor. Eleven days after infection rabbit RH died very suddenly. Blood smears showed the presence of B. besnoiti trophozoites. On the same day swelling of the hind limbs was noted in rabbit RB. The following day the swellings also appeared at the base of the ears. The blood smears were positive. The animal was killed in extremis, and a further three rabbits were injected in order to maintain the Fuls B. besnoiti strain by serial passage. Observations on the maintenance of the strain are listed in Table C of the Appendix. Rabbit RT had a more prolonged incubation period which was followed by a more pronounced generalised oedematous swelling, and a large number of parasites in the blood as seen on two consecutive days. The parasites were morphologically identical with those encountered previously in cattle.

In order to fulfil Koch's postulates, 13.0 ml. of blood from the latter rabbit killed in extremis, was injected into an ox (No. 5887). The animal developed fever accompanied by mild clinical symptoms, and 23 days later cysts were seen in skin sections.

Having determined that transmission can be effected readily in rabbits by means of infective blood, attempts were made to establish whether the disease can also be transferred by means of either serous fluid present in the oedematous swelling of the subcutis or suspensions prepared from liver and spleen of diseased animals. Serous fluid, obtained from the pronounced oedematous swelling at the base of the ears of rabbit No. 13, was injected subcutaneously into a susceptible rabbit. The quantity used was 5.0 ml. The recipient developed a typical reaction accompanied by a microscopic blood infection and died eleven days later. The intraperitoneal injection of 5.0 ml. of a suspension prepared from the spleen of rabbit No. 1305, into rabbits Nos. 1308 and 1309 was followed by a typical reaction in both animals. These experiments thus showed that transmission is possible by means of materials other than blood.

Earlier in this discussion it has been stated that in the case of cattle cohabitation between diseased and susceptible animals is not followed by transmission. In order to verify this statement an attempt was made to infect rabbits using either an infected suspension prepared from the liver and spleen or a mixture of faeces and urine from actively affected rabbits, and administering these materials by the oral route. A stomach tube was used for the administration. Two rabbits (Nos. 1660 and 1661) each of which had received 10.0 ml of the spleen and liver suspension failed to react. On challenging their immunity one month later they proved to be fully susceptible. Two controls (Nos. 1658 and 1659) that had received 5.0 ml. of the same organ suspension intraperitoneally both contracted besnoitiosis and died. Although these tests have shown that infection by the oral route is not followed by transmission, attempts were nevertheless made to infect rabbits by means of excreta from actively infected animals. For this purpose 20.0 ml. of a suspension of faeces in urine was given to two rabbits (Nos. 1654 and 1657) by the oral route. Both animals were observed for a month without showing a reaction. The experiment was thereupon repeated on another two rabbits (Nos. 1675 and 1676) each of which received 30.0 ml. of an excreta suspension per os. Both failed to react, and on challenging their immunity one month after infection they proved to be fully susceptible.

Consideration of these results makes it apparent that transmission does not result by the oral route even when the organ material used contained active B. besnoiti parasites. Cohabitation between actively affected and susceptible animals should, therefore, not be followed by transmission.

C. Period of infectivity: The experiments of Cuillé and Chelle (1936), and the observations recorded previously, indicated that the infection can only be transmitted artificially by means of blood from cattle during the primary stage of the disease. Experimental work was not done on either cattle, sheep or goats to determine critically, by means of repeated subinoculations of blood, the period of infectivity during this stage. One incidental observation may be recorded here indicative of a very brief infective period. Blood from heifer No. 6839, collected on the

/57.....

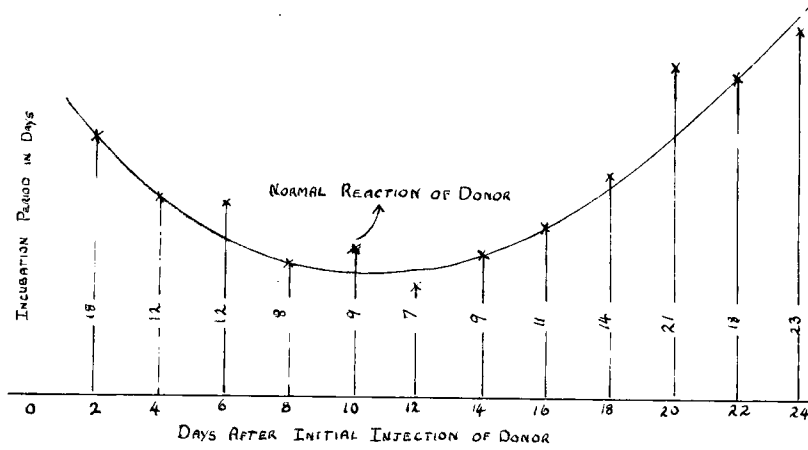
day when the peak of the temperature reaction was obtained, proved to be infective for a rabbit. This peak occurred two days after the initial rise of temperature. The sub-inoculation was repeated five days later. By that time the temperature had subsided to  $104^{\circ}\text{F}$ , and the cutaneous oedematous swelling had just begun to develop. None of the four rabbits used for the second subinoculation showed any reaction. Their immunity was challenged two months later and all proved to be fully susceptible.

As the rabbit had proved a pre-eminently useful laboratory subject, the following experiment was conducted to determine the duration of the period of infectivity of rabbit blood after an artificial infection. Subinoculations were performed from an infected donor by injecting 5.0 ml. of its blood intraperitoneally into one susceptible recipient two days after the date of infection of the donor, and repeating this process every alternate day for 24 days. The donor reacted on the fifth day by elevation of temperature. On the tenth day the peak of fever was reached, and trophozoites could be demonstrated in the blood. In the normal course of events this would have been the time selected for subinoculation. All twelve recipients reacted thus indicating that the donor's blood had been infective during the total period under test i.e. throughout the incubation period, and up to 14 days after the peak of reaction. Another rabbit, which had been injected 47 days previously was used as donor 30 days after the first appearance of parasites in the blood. Two susceptible rabbits received 5.0 ml. of blood intraperitoneally. No reaction resulted. The immunity of one of the recipients was challenged two months later and proved to be fully susceptible. The other rabbit died from an unknown cause.

Consideration of these results makes it apparent that the duration of the period of blood infectivity, as determined in a single rabbit (24 days), is considerably longer than that observed in a single heifer. It is, however, impossible to conclude from these tests whether or not the end point had been determined accurately for both species of animals.

An interesting fact emerged from the first mentioned rabbit experiment. The length of the incubation period in the positively reacting recipients increased proportionately with the time interval elapsing between transmission from and peak of infection of the donor. This may be illustrated

graphically as follows:



Graph illustrating the difference in length of the incubation period.

EPIZOOTOLOGY

The present state of the knowledge of the epizootology of bovine besnoitiosis is far from complete as the natural mode of transmission is obscure. Observations made in Southern Africa, during the last two decades, have shown that cyclical variations in the incidence of the disease do occur, and that they are similar to those seen in horse-sickness, bluetongue, three day sickness and sweating sickness. General experience has been that a large number of cattle contract besnoitiosis in December, January, February and March, while only sporadic outbreaks occur in autumn, winter and spring. Observations made in other enzootic regions of Africa and Europe have also shown that the highest incidence is in summer. The presence of susceptible cattle determines the degree of prevalence.

In Transvaal the distribution extends widely into the bushveld and bankenveld areas with an altitude of up to 1200 metres above sea level, and an average rainfall of 600 mm. per annum. The natural water supply on the infected farms varies considerably. Some farms border on perennial rivers, others are situated along rivulets containing running water only during the summer rainfall season, while the remaining farms depend mainly on a subterranean supply of water or alternatively on water collected in dams.

The source and maintenance of bovine besnoitiosis in nature still needs to be determined. This becomes evident if one considers the sporadic outbreaks of equine besnoitiosis. Field observations revealed in a single affected horse in the Grahamstown district in the Eastern Cape Province, and ten cases in the Vryheid and Umvolozzi districts in Natal (vide supra - Distribution, Table I). The source of infection is mysterious if one considers the fact that neither before nor after the outbreaks had cases of equine besnoitiosis been detected. Consideration of these events suggests that an animal other than a soliped may have served as a reservoir. It thus becomes apparent that a clear conception of the epizootology will only become available when the natural mode of transmission has been determined.

## PATHOGENICITY

A. Susceptibility to natural infection:- Extensive observations by numerous investigators on the incidence of bovine besnoitiosis, listed in Table I, have shown that this disease has so far only been encountered in cattle. From field surveys conducted in Transvaal it appears that stock older than 18 months are highly susceptible, while the course of the disease, as observed in a few younger animals, is relatively mild. Attempts to establish the degree of susceptibility of cattle did not entertain much hope when it became apparent that the five artificially infected adult animals listed in Table B of the Appendix developed either mild or inapparent reactions. Consideration of these results indicated, that for studies on the pathogenicity of B. besnoiti in different age groups, a very large number of animals would have been required, and even then it would have been questionable whether observations on artificially infected cattle are comparable with those described from natural cases.

When an outbreak occurs in a herd only a relatively small number of animals show clinical symptoms. There is no difference in susceptibility between different breeds and animals of either sex; the indigenous Afrikander breed is just as susceptible as the exogenous breeds, Hereford and Short Horn. Although the mortality rate does not exceed ten per cent, the disease is nevertheless responsible for severe economic losses. Affected animals lose condition, milk production decreases considerably, cows may abort, bulls often become sterile, and hides are valueless for tanning. (Pols, 1954 a).

The relatively small number of animals that become visibly infected in any one season, even in large herds, gives rise to speculation. Of the theoretical possibilities, the only one at present worthy of serious consideration is that of clinically inapparent infections in very young and possibly also in some of the mature stock with a consequent state of premunition. The fact that susceptible animals, newly imported into an enzootic region, often contract the disease during the first besnoitiosis season seems to support this theory.

/60.....

B. Susceptibility to artificial infection:-

(a) Attempts to infect laboratory animals:-

(1) Actively affected cattle used as source of infection:-

The successful transmission of B. besnoiti to rabbits by means of blood derived from ox No. 6840 and heifer No. 6839 has already been described. At the time when the susceptibility of rabbits was established an attempt was also made to transmit the infectious agent to guinea pigs, rats and mice. As none of these laboratory animals developed a clinical reaction after the intraperitoneal injection of blood from ox No. 6840 it was assumed that they were not susceptible.

(2) Actively affected rabbits used as source of infection:-

Inoculation of 2.0 to 3.0 ml. infected rabbit blood either subcutaneously or intraperitoneally into 16 guinea pigs resulted in temperature reactions of up to 106°F appearing in ten cases. The incubation period varied from 4 to 17 days. Isolated trophozoites were found in blood smears in two cases 8 and 15 days after infection. In a third guinea pig the presence of parasites was determined by a biological test on a rabbit. Several serial passages in guinea pigs were conducted, but these animals developed nothing more than an irregularly intermittent temperature. These tests showed that although the guinea pig is susceptible it is not suited for laboratory tests.

Further transmission experiments showed that no demonstrable reactions, as judged by pyrexia, symptoms and blood smear examinations was obtained after injecting infective rabbit blood into mice, rats, hamsters, fowls and a rock dassie (Procavia capensis Pallas). The observations are listed in the subjoined Table II.

/61.....

TABLE II.

Observations on Artificially Infected Laboratory Animals.

Species	No. injected	Quantity of blood in ml.	Route of infection	Result	Smear examination.
Mice	16	1.0 to 2.10	ip. or sc.	N.R.	Negative
Rats	6	2.0	ip. or sc.	N.R.	Negative
Hamsters	2	2.0	ip. or	N.R.	Negative
Dassies	4	4.0	ip.	N.R.	Negative
Pullets and Cockerels	8	2.0 to 3.0	ip., sc., iv.	N.R.	Negative
Adult hens	6	3.0 to 5.0	ip, sc., iv.	N.R.	Negative
Adult cocks	2	3.0 to 5.0	sc., iv.	N.R.	Negative

N.R. = No reaction.

These animals were observed for a period of six weeks. No attempts were made to ascertain whether any of them had developed an inapparent infection. The tests nevertheless showed that these animals are not suited for laboratory tests.

(b) Attempt to infect sheep, goats, horses and a dog:-

(2) Actively effected rabbits used as source of infection:-

(i) Susceptibility of sheep and goats: For these experiments three rams and one ewe and four billy goats, which had been raised in a non-enzootic bovine besnoitiosis area, were employed. The observations on these animals are detailed in Table C of the Appendix. Sheep (No. 86569) and goat (No. 79328) each received 350 ml of rabbit blood by the intravenous route.

After an incubation of seven days the sheep showed pyrexia and anorexia for several days. No parasites could be demonstrated in blood smears but they appeared in two rabbits used for the biological test. The immunity of this animal was challenged 14 months later. It proved to be solidly immune as no reaction was noted over a period of four months. In contradistinction to the sheep the goat showed distinct clinical symptoms. The temperature started to rise six days after the artificial infection. Two days later the animal appeared ill and showed swelling of the nose. After four days the face and anterior portion of the neck became oedematous, eyes showed lachrymation, and from the nose a mucoid discharge appeared. The animal was disinclined to

move, and anorexia persisted for a week. The temperature rose to a peak of 107°F 15 days after infection. Blood smear examination failed to reveal any parasites. In two rabbits, that received 5.0 ml. blood intraperitoneally, B. besnoiti could be demonstrated in the blood filus. The subcutaneous swellings subsided a fortnight after their onset, and a week later young cysts were found in the skin sections. For three months thereafter a slight diffuse alopecia was noticeable over the anterior half of the body. Skin lesions, so striking in bovine besnoitiosis, never appeared. There was considerable loss in condition. Gradual recovery ensued during the following five months.

Serial passage of B. besnoiti in sheep and goats was next attempted. Sheep (No. 89632) and goat (No. 33537) each received 30.0 ml. blood from rabbit No. 1468. Both animals showed no symptoms other than pyrexia. In the goat cysts could be demonstrated in the skin three- and - a-half months after the artificial infection. In both animals the presence of B. besnoiti was determined by biological tests on rabbits. Blood from the sheep and goat was collected at the height of the febrile reaction, and 50.0 ml was injected intravenously into a sheep (No. 615) and a goat (No. 88549), both of which developed pyrexia after six and four days respectively. Biological tests conducted on rabbits showed that both the sheep and goat harboured B. besnoiti. The serial passage was continued for only one further generation in a sheep (No. 145) and in a goat (No. 88536). Again 50.0 ml blood collected at the height of the temperature reaction was used as inoculum. The recipients showed pyrexia a week later. B. besnoiti could not be demonstrated in the daily blood smears of the sheep and goat but appeared in the rabbits used for the biological test.

Consideration of these results makes it apparent that although sheep and goats are susceptible to B. besnoiti, the serial passage in these animals did not enhance the virulence of the parasite. Trophozoites could not be demonstrated microscopically in blood smears from these animals even though cutaneous cysts were detected in two of the goats.

(ii) Susceptibility of the horse and dog:- The two horses and the dog used for these tests had been reared in a stable kept under tick-free conditions at Onderstepoort,

/63.....

and had never been exposed to bovine besnoitiosis. The inoculum consisted of a pooled spleen and liver suspension and/or blood. The horses each received 30.0 ml. of the organ suspension and 30.0 ml. blood intravenously, while the dog received 10.0 ml. by the same route. The two rabbits, used for the serial passage of the B. besnoiti strain, served as controls. Both reacted promptly to the disease. No demonstrable reactions were seen in either the horses or the dog over a period of three months. As no biological tests were conducted on rabbits, it is impossible to state whether or not an inapparent form of the disease had occurred.

Consideration of the studies on the pathogenicity makes it apparent that besides cattle, susceptibility to B. besnoiti has also been established in sheep, goats, rabbits and guinea pigs. Critical experiments, based on biological tests on rabbits, will have to be conducted to determine whether or not mice, rats, hamsters, fowls, dassies, horses and dogs develop infection. It is also recommended that studies on the pathogenicity be extended to include wild animals as they may disclose unknown reservoirs of B. besnoiti, serving as a source of infection in nature.

### PATHOGENESIS

The course and pathogenesis in bovine besnoitiosis follows the general pattern of most infectious diseases in as much as there is an acute phase with parasitaemia, fever and general symptoms of malaise, followed by localisation in a particular tissue, and consequently a more chronic phase ensues. It is unique in this respect, that the tissue involved is the more superficially situated connective tissue, i.e. the dermis, subcutaneous tissue, fascia, as well as the connective tissue of the scleral conjunctiva, nasal and laryngeal mucous membranes. More deeply situated connective tissue is very rarely parasitised and then only to a slight degree. Although clinically complete recovery may follow mild attacks of the disease, one cannot really speak of a complete restitution, as a certain degree of scleroderma, however slight, undoubtedly persists, as do cysts containing viable organisms.

/64.....

Mortality, directly attributed to B. besnoiti infection only, is low. Few such cases were seen by the author. References to such cases in the literature are not clear enough to allow critical evaluation on this point. If death occurs, it will often be due to early or late complications. Of the former, secondary infection through the severely affected and rapidly developing decubital lesions as well as the result of deranged physiology of the skin (massive exudation) and possibly the effect of the toxin produced by the parasite, are the most important factors.

In the rabbits, on the contrary, uncomplicated deaths during the acute phase of the disease are fairly frequent. It is considered that the anaemia which is such a prominent symptom in the rabbit, plays an important rôle in this respect. It should also be borne in mind that blood parasitaemia in the case of the rabbit is much more pronounced.

Cattle surviving the acute phase do not die as a rule although severe cases waste away and remain unthrifty and therefore more subject to late complications. In rabbits more deaths have been experienced, but here inter-current infections, a condition inherent in captivity, form complicating factors that are impossible to analyse accurately.

Cutaneous manifestations are not clinically apparent in the rabbit after the primary stage of the disease. Compared with the heavy infection in the blood stream, the number of cysts found in the skin is comparatively low, especially when one considers the rarity of parasites in the blood circulation of cattle and the subsequent density of infection often found in the skin.

The ensuing immunity was shown to exist in all cases tested, in cattle from 7 months to 3 years, in sheep from 5 to 18 months, in one goat after 5 and 18 months, and in rabbits from 70 to 112 days after date of initial infection. The view has already been advanced that the nature of the immunity is one of premunition or immunatus non sterilisans, as cysts with viable organisms could be

/65.....

demonstrated in cattle 10 years after infection, and thus are presumed to remain for life.

The deleterious effect of B. besnoiti in spermiogenesis is an interesting and important finding. Its temporary presence forms a useful indication in laboratory experiments, and is of considerable importance to the cattle breeder and clinician. Although exact information on spermiogenesis in most other infectious disease is lacking, nevertheless it would appear that sperm production is interfered with to a relatively more severe degree in besnoitiosis when compared with the severity of clinical symptoms. Except for the occasional cyst in the connective tissue in the testis these organs are not grossly affected. Circulating parasites are few and far between in the acute phase and absent subsequently. Hence influences other than the direct presence of parasites or fever must affect the testes.

#### SYMPTOMATOLOGY

##### A. Besnoitiosis in cattle, sheep and goats.

The first account of a disease in cattle, referred to as "l'elephantiasis et de l' anasargue de boeuf", was published by Cadéac (1884). In subsequent publications Besnoit and Robin (1912, 1914) gave a lengthy account of the malady. The description of the symptoms was accompanied by photographs of affected cattle and cutaneous cysts harbouring the causal agent. Further records on the symptomatology in naturally infected convalescent cases were published by Franco and Borges (1916), Bennett (1913), Hofmeyr (1945) and Pols (1954 a), while the course of the disease in artificially produced infections was given by Cuillé, Chelle and Beruleau (1936), Cuillé and Chelle (1937), Barrairon (1938) and Pols (1954b).

During the course of the present investigations observations were made on 50 affected cattle. Of these three were submitted at Onderstepoort from the field when they showed the febrile phase of the disease, seven were produced artificially, and the remainder were chronically affected natural cases. The five sheep and five goats, included in these studies, were all artificially produced cases.

Field surveys in the Northern Transvaal by the investigators, listed in Table I, have shown that severe cases, exhibiting the full range of the symptoms, occur relatively infrequently. The writer only observed one very severe case (bull No. 6851, Table B of the Appendix) during the whole period of illness which terminated fatally. From observations on experimental cases in cattle (Tables B and G of the Appendix) one may conclude that the incubation period varies from six to ten days, and that pyrexia persists for three to six days. As a rule the temperature fluctuated between 103°F to 107°F. Anorexia, depression and listlessness constituted accompanying signs. Photophobia was usually a distinct symptom, as was the hot, painful subcutaneous oedematous swelling affecting any part of the body but with a predilection for one or more limbs, and/or pendulous regions (dewlap, scrotum and flank) at/or shortly after the peak of the febrile reaction. Except in severe cases, the swelling, although in itself diffuse, remained restricted to a particular region or regions. The swollen area became indurated as the disease progressed. The enlargement of lymphnodes, which was a common symptom in the primary stage of the disease and of diagnostic value, was present to a variable degree. In experimentally produced cases, however, it was moderate or absent. That cases do occur with virtually no fever, and no anasarca or any other symptoms, commonly seen in the primary stage, was exemplified by cow No. 6793 (Table B of the Appendix). The extent and severity of the skin lesions in the second and third stages of the disease varied widely, more or less proportionally to the preceding swelling. The reactions in the four experimentally produced cases (Nos. 6839, 5895, 5887 and 5870, Table B of the Appendix) were mild. In two cases only pyrexia was seen, and in the remaining two animals fever was accompanied by subcutaneous oedematous swellings of the limbs. At the height of fever the animals were listless and showed inappetance. Permanent skin lesions were not prominent.

Regarding the course of the disease it may be stated that in severe cases the secondary depilatory stage is the critical phase on which the prognosis is based. The advanced skin lesions with folding and cracking and serous-sanguinous exudate form a favourable port of entry for secondary bacterial infections, which is the factor often

responsible for death. The clinical syndrome then becomes one of a bacteriaemia.

Animals surviving this phase usually do not die but progress to the third or seborrhoea sicca stage (Hofmeyr 1945). The severely affected cases remain emaciated for months on end. For all practical purposes such animals are poor doers, and represent a total economic loss. Although one may speak of recovery in the sense that active inflammatory processes are no longer present, the skin remains thickened and wrinkled for life. A certain amount of hairgrowth will again occur. In such skin the cysts are found studding the cutis vera, and are present to a lesser extent in the subcutis, superficial fascia, without evidence of any inflammatory reaction. A very slight skin infection produces no clinically visible skin pathology. The chances of encountering cysts in the skin pathology. The chances of encountering cysts in skin biopsies, from such cases, would be rare indeed.

Cysts, which appeared structurally normal and harbouring viable parasites, remained unaltered, at the stites mentioned, for the rest of the animal's life. Ten years after the original infection the author still found cysts. It is in this sense that the statement "recovered animals remain carriers for life" was made by Pols (1954 a). There was no evidence that the cysts ruptured to liberate their contents on the surface of the skin or discharging them into the host's blood circulation.

The impression was gained that the pendulous parts and loose folds of the skin formed predilection sites for the development of pathological changes, namely in the flankfold, scrotum, dewlap and eyelids, thus corresponding to the areas which were swollen during the primary stage of the disease. The only exception to this correlation was found in the limbs, where clinically visible changes generally, were by no means as striking as the preceding swellings. No systematic investigations on the differential distribution of cysts over the whole cutaneous area were undertaken.

Clinical observations which had shown that B. besnoiti cysts often appeared in the sclera of naturally affected cattle (Hofmeyr, 1945), raised the question whether this phenomenon could be used as a reliable method for making a diagnosis. For the evaluation of this symptom as a diagnostic method, observations were made on fifteen natural cases of typical chronic bovine besnoitiosis at the Municipal Abattoir of Pretoria. In nine of these animals distinct white nodules could be seen macroscopically and microscopically in the sclera and skin sections, four animals showed indistinct nodules not of parasitic origin, as determined microscopically, and in two cases no globules were demonstrable even though skin sections revealed the characteristic parasitic cysts. In mild natural as well as in experimentally produced cases they were frequently not apparent upon visual examination.

At autopsy no internal pathological lesions, directly referable to besnoitiosis, were observed with the exception of cachexia and the presence of cysts, visible as opaque, whitish, raised nodules (0.5mm. in diameter) in the mucous membrane of the upper respiratory tract, and that of the trachea up to its bifurcation into the bronchi, and in the cutis, subcutis and aponeuroses of the muscles. No cysts were ever encountered in mucous membrane of either the abomasum, or small and large intestine. An occasional cyst was found in the capsule of the spleen, in the connective tissue around the pampiniform plexus and in the tunica albuginea of the testis.

Clinical symptoms exhibited by the four artificially infected sheep did not present any characteristic features. Pyrexia and anorexia for a few days were the only observable symptoms. Fever persisted for six days, rising steeply to a peak of about 107°F, and then subsiding gradually to normal. The only pregnant sheep, of this group, aborted eight weeks after the height of reaction. No parasites could be demonstrated in blood smears of any of the sheep nor could cysts be found in skin section. The presence of B. besnoiti infection could only be proved by subinoculation into rabbits at the peak of the febrile reaction and by subsequent immunity to challenge.

In the four experimentally infected goats the temperature was essentially similar to that in sheep. They appeared visibly ill and were listless and dejected. As described in detail previously (page 61), one goat No. 79328) reacted severely with oedematous swelling of the face and neck, lachrymation, mucoid nasal discharge and dyspnoea. This was the only small ruminant that showed cutaneous lesions. No parasites were found in blood smears but cysts were demonstrable in skin sections in two goats.

Economically one of the most important symptoms, namely sterility, must be singled out for special consideration. It had been observed that bulls naturally infected with B. besnoiti developed a partial or complete sterility. It was often permanent. Out of nine pure-bred Hereford bulls set to this Institute from the Northern Transvaal, seven years previously on account of besnoitiosis and sterility, two (Nos. 4197 and 4201) were available for the study on the sperm picture.

Bull No. 4201 ejaculated 8.0 ml. of a cloudy fluid in which no sperm were present. On one occasion bull No. 4197 ejaculated 8.0 ml. of milky semen. Sperm motility was good, but there were a large number of loose heads. Ten months later 7.0 ml. of a milky semen was obtained, and although the motility was good only 35 per cent of the sperms were alive, 12 per cent had loose heads and 8 per cent coiled tails. The fertility of this bull was thus permanently impaired.

Studies on the sterility in besnoitiosis were extended to include two bulls (Nos. 5981 and 5873), one ram (No. 1952) and one billy goat (No. 3171) which were infected artificially by using infective rabbit blood as inoculum. Table G of the Appendix. In all instances semen samples were obtained by electrical ejaculations before and after infection.

Before infection it was determined microscopically that the sperm-picture of the two bulls was almost identical. A count of 65 per cent (bull No. 5981) and that of 68 per cent (bull No. 5873) was recorded. Sperm motility in both cases was good. Semen samples were taken

/70.....

subsequently at approximately weekly intervals for the first eight weeks, and thereafter at roughly monthly intervals until about seven months had lapsed since the date of infection. Three days after artificial infection the sperm picture was not materially altered. A week to a fortnight later no sperm were present. This picture was only modified by the appearance of some dead sperms at occasional intervals until after 137 days when bull No. 5981 had 27 per cent live sperms together with all types of abnormal forms in the semen. A month later considerable improvement had occurred, and by the 202nd day the normal sperm picture was restored. Bull (No. 5873) only began showing 12 per cent live sperms on the 173rd day. The last examination was done on the 227th day, when 55 per cent live sperms with fair motility were present.

It must be stated that three weeks after infection bull No. 5981 developed a Corynebacterium abscess in the left testicle which became fibrotic. Despite the adverse systemic reaction due to this infection, good recovery of the sperm picture eventually took place on the 202nd day after infection. The other bull (No. 5873) suffered from an intercurrent Trichomonas foetus (Riedmüller 1928), infection 42 days after the artificial B. besnoiti transmission. It is considered that the former infection did not modify the effect of the latter on the sperm picture in any material way. The immunity of this bull was challenged with a second injection of infected rabbit blood. The injection was followed by severe shock but no further reaction occurred. The sperm picture was not influenced in any way, and during the ensuing eleven weeks T. foetus was not detected.

In the case of the ram and billy goat it was determined that before infection the sperm picture was normal. A count of 80 per cent (ram No. 1952), and that of 76 per cent live sperms (goat No. 3171) was recorded. After infection semen samples of the former animal were examined eleven times over a period of four-and-half-months, and those of the latter eight times over a period of three-and-a-half months. The sequence of events followed the same pattern in the small ruminants as in the bulls but took place within almost half the span of time. Within a fortnight the excellent

/71.....

sperm picture, obtained before infection, had deteriorated markedly, and within four weeks no sperms or at least no live ones could be seen. Approximately two-and-a-half months later live sperms again appeared in the ram (30 per cent) and goat (49 per cent). Complete recovery was observed within four-and-a-half months in the ram, and within three-and-a-half months in the goat. Both animals did not react when their immunity was challenged one month after recovery. Their sperm-picture was not affected.

#### B. Besnoitiosis in rabbits.

In rabbits clinical symptoms during the febrile stage were more prominent than in either cattle, sheep or goats under experimental conditions. A well-defined thermal reaction developed in nearly all rabbits. The fever persisted for three to seven days, and ranged between 103°F to 107°F, mostly between 105°F and 106.6°F. During the fever animals became listless, refused to feed and lost weight rapidly. At about the peak of reaction, hot, painful, subcutaneous oedematous swellings, especially of the ears, head, limbs and scrotum developed in the majority of cases. In some males the swelling progressed to a complete necrosis of scrotum and testes. Occasionally the swellings extended over the whole body. A few does developed an oedematous swelling and hyperaemia of the vulva. These inflammatory oedemas lasted from three to fourteen days.

In contra-distinction to cattle the skin lesions did not progress. The swelling subsided, and neither proliferative thickening of the skin, serous-sanguinous exudate nor the seborrhoea sicca stage was ever observed. This may have been due to the fact that mortality in rabbits is so much higher, and only those with relatively light symptoms survived.

A leucocytosis accompanied by anaemia was a characteristic symptom (Pols, 1954 b). Differential counts revealed from 20 to 70 per cent monocytes. The rise in the monocyte count occurred concurrently with the rise in temperature. Even from ordinary blood smears the monocytosis often could be inferred readily. Similarly, the

monocytosis declined more or less with the drop in temperature.

At about the peak of reaction anaemia became apparent in blood smears. Hypochromatic macrocytes were the first to appear. Subsequently polychromasia, commonly seen in normal rabbit blood, became more pronounced, and often punctate basophila developed. In many cases there was as much as a 50 per cent or even 70 per cent drop in the number of erythrocytes. To illustrate the variation in the blood picture an artificially infected rabbit No. 330, with an incubation period of 11 days, two days later showed an erythrocyte count of 1.4 million, and a leucocyte count of 4,400 per cmm. After another four days the count changed to 1.22 million and 14,300 respectively. A second artificially infected rabbit No. 321 had an incubation period of 8 days. Five days later this animal had an erythrocyte count of 2.64 million and a leucocyte count of 7,800 per cmm. After four days the figures changed to 3.14 million and 5,200 respectively. At autopsy it was observed that the emaciation in both animals was associated with anaemia and ascites.

The duration of the incubation period varied from 3 to 15 days with an average of 7 days. In fatal cases the duration of the disease varied from 2 to 16 days with an average of 6 days. In one instance death occurred 38 days after the commencement of fever.

During the early stages of the serial passages of B. besnoiti it was noticed that the symptoms were more pronounced than at higher levels of passage. This was illustrated by comparing the observations made on 22 rabbits used for generations Nos. 20 to 25 with those on animals employed for generations Nos. 120 to 125. In the first group 2 rabbits died from unknown causes, 7 were killed for the collection of infective blood, ten died from besnoitiosis, and three recovered. In the second group one rabbit died from shock immediately after infection, seven were killed for the collection of infective blood, and ten recovered completely. Depending upon the severity of the reactions survivors, which had suffered severely,

/73.....

took anything from one to three weeks to recover. Generally speaking less severe reactions, and consequently a more rapid recovery were seen more often in rabbits at higher levels of the serial passages. Of all rabbits used 15 per cent did not show a parasitaemia, even though they developed either fever and/or a moderate oedema of the subcutaneous tissues. Over the last five generations the percentage rose to 40 per cent. These observations thus support the opinion, expressed by Pols (1954 b) that the disease took a more protracted and less severe course as serial passages progressed.

Although it was established that the genitalia became affected, no work was conducted to evaluate the effect of B. besnoiti on the reproductive ability of both sexes of rabbits. It may be mentioned in passing that a doe (No. 108) gave birth to a litter of seven rabbits two months after the date of infection and seven weeks after the commencement of the disease. The whole litter died within 24 hours. Examination of blood and organ smears, as well as biological tests on rabbits, failed to reveal B. besnoiti in the offspring. Since conception had taken place a fortnight after subsidence of the clinical symptoms, and as no parasites were seen in the offspring, no conclusions could be drawn whether or not the deaths were a sequel to besnoitiosis.

At autopsy, rabbits dying from besnoitiosis within a few days after commencement of symptoms, only revealed subcutaneous oedema, ascites and sometimes subepicardial petechiae. Those surviving for longer periods showed, in addition emaciation and anaemia. Apart from occasional lesions due to intercurrent infections, such as pneumonia, coccidiosis, and non-specific enteritis, pathological changes, not directly referable to besnoitiosis, were seen from time to time. These included either hydrothorax and/or a considerably enlarged gall bladder. The mucous membrane of the stomach and intestine never contained parasites. Macroscopically no nodules, as seen in cattle, could be detected in the cutis, subcutis, fascia, sclera, and nasal, pharyngeal, laryngeal and tracheal mucous membranes.

/74.....

## DIAGNOSIS

Although a tentative diagnosis of bovine besnoitiosis can be made by considering the epizootology, the clinical symptoms and the lesion at autopsy, definite diagnosis depends upon demonstrating B. besnoiti microscopically in either blood or tissue smears, and in sections prepared from either the cutis, subcutis, sclera or mucous membrane of the upper respiratory tract.

Epizootologically, bovine besnoitiosis is restricted to certain regions. When investigations are being made it is essential to determine the local incidence of this malady, and other diseases likely to be confused therewith, and the origin of the affected animals. These considerations should guide the investigational procedure.

During the commencement of the thermal reaction of besnoitiosis, a definite diagnosis may present difficulties, as no specific symptoms may be evident. During this period microscopic examination of blood and lymphnode smears may reveal the infectious agent. Failing this a biological test on rabbits, using blood from the suspect, should be undertaken. Although this is rather a lengthy procedure, it is the only reliable method of diagnosing besnoitiosis during the primary stage of the disease. When applying this test due consideration should be paid to the viability period of the causal agent in vitro (vide supra - Aetiology, Subsection (f)).

As the disease progresses the possibility of making a diagnosis improves. Careful examination of sections prepared from skin biopsies usually reveal Besnoitia cysts at different stages of development.

When the patient enters the clinically recognizable phase of the disease no difficulty should be experienced in making a diagnosis. Visual examination will reveal cysts in nasal mucous membrane, and in many instances also in the sclera. Spores can be demonstrated in deep skin scrapings, and in parasitized cysts in sections prepared from skin biopsies.

/75.....

TREATMENT

As far as could be ascertained from the literature only a few attempts have been made to treat bovine besnoitiosis. Leitao (1948) employed three injections of 30.0 ml. of a ten per cent solution of formalin at three daily intervals for the treatment of chronically affected mature cattle. Herin (1952) advocated a single intravenous injection of 30.0 ml. of a one per cent solution of formalin during the primary stage of the disease. For chronically affected adult animals he recommended intravenous administration of 20.0 to 40.0 ml. of Lugol's iodine at four to seven daily intervals. Five injections were usually sufficient. Although no critical evaluation of the results were given both investigators expressed the opinion that these forms of treatment had a beneficial influence on the course of the disease.

The procedures instituted during these studies involved the use of protozoocidal and bactericidal drugs. Observations on the artificially infected and treated rabbits, and the chemotherapeutic agents employed are detailed in Table H and I of the Appendix, and are summarized in the subjoined Tables IV and V respectively in the text. Observations on rabbits comprised recording the body temperature twice daily, examining blood smears daily during the period of the thermal reaction, and noting the appearance of the subcutaneous oedema of the ears, limbs and external genital organs. The route of administration, the dose per Kg. body weight, the number of treatments given, and the termination of the disease are also listed in the appended tables.

Two series of experiments were conducted. In the first group 53 rabbits of the lower passage levels during 1954, and three of the higher passage levels during 1956 (pentamidine treated rabbits), were treated once or several times during the period of the febrile reaction. The second group, comprising 26 rabbits of the higher passage levels, during 1956 and 1957, were treated during the incubation period and in 20 of them treatment was continued for either one or more days during the reaction period.

(a) Treatment during the period reaction:- It will be seen from Table H that all 56 rabbits showed a febrile reaction. The incubation period varied from 5 to 15 days. The duration of the disease, which varied from 3 to 15 days, was, with the

exception of one rabbit (No. 96 - 52 days), within the usual limits seen in untreated animals (vide infra - Table C of the Appendix). A microscopic infection of B. besnoiti was observed over a period of one, two, three or four days in 50 rabbits. A subcutaneous oedema developed in 41 animals.

In assessing the value of the chemotherapeutic agents, two factors, namely, a possible variation in either the morphology or virulence of the parasite were taken into account. No morphological changes in the parasites suggestive of a specific action by any of the drugs, was observed. If the recovery rate is accepted as a criterion for the specific action of the drugs used, then only the effects of sulfamerazine and pentamidine need be considered.

A total of 19 rabbits were treated with sulfamerazine, and of these 6 survived (= 26.3 per cent). Of these four received a total of 400 mgm (one recovery), two 800 mgm., one 900 mgm., four 1200 mgm. (one recovery), one 1500 mgm., three 1600 mgm. (one recovery) and four 2000 mgm. (three recoveries). Should sulfamerazine have been responsible for the recoveries, then one would have expected that with the increase of the dosage, the recovery rate should have increased accordingly. Although it appears that this has been the case, a definite conclusion on the efficacy of sulfamerazine cannot be drawn as the number of rabbits employed for dosages lower than 2000 mgm., was inadequate. The three rabbits, used for screening pentamidine as a cure, were derived from the higher B. besnoiti passage levels when the virulence of this parasite had decreased for the rabbit. Although two out of the three treated rabbits survived, the recovery is attributed to the decrease in the virulence of the parasite rather than to the specific action of pentamidine.

/77.....

TABLE IV  
TREATMENT DURING THE PERIOD OF REACTION

Drug	Number treated	Severity of disease	Number that died
Achromycin and Phenergan	6	1 (+) 5 (+ +)	1 4
Aureomycin	4	2 (+ +) 2 (+ + +)	2 2
Babesin	2	1 (+ +) 1 (+ + +)	1 1
Gonacrin	2	2 (+ + +)	2
Pentamidine	3	3 (+ +)	1
Phenamidine	5	2 (+) 3 (+ + +)	0 3
Plasmoquin	1	1 (+ +)	1
Sodium Iodide	2	1 (+ + ) 1 (+ + + )	1 1
Sulfamerazine	15	2 (+) 12 (+ +) 1 (+ + +)	0 8 1
Sulfamerazine and Phenergan	4	4 (+ +)	4
Sulfamezathine	12	2 (+) 9 (+ +) 1 (+ + +)	2 7 1

+ = mild  
 + + = severe  
 + + + = very severe

(b) Treatment during the incubation period, and in a large number of cases also during the period of reaction:- It will be seen from Table I that all the 26 rabbits showed a febrile reaction. The incubation period varied from 5 to 16 days. The duration of the disease (5 to 11 days) was within the usual limits seen in untreated animals of the

/78.....

higher passage levels. A microscopic infection of B. besnoiti was observed over a period of either one or two days in 21 rabbits. A subcutaneous oedema appeared in 12 animals.

In assessing the action of the various drugs several facts became apparent. The duration of the incubation period was not prolonged unduly. No morphological changes in the parasites, suggestive of a specific action of any of the drugs used, was seen. The degree and duration of the microscopic parasitaemia was less than that observed in the previous group of rabbits. The over all mortality rate (36 per cent) was much lower than in the previous group (76.7 per cent). This reduction is ascribed to the decrease in the virulence of B. besnoiti following the serial passages in rabbits (vide supra - Symptomatology, page 72), and not to the action of any of the drugs used. Rabbits with three exceptions, used for the former experiment (Table IV), were derived from the low passage levels during 1954. Animals used for this study (Table V) were obtained from the higher passage levels during 1956 and 1957. It thus became apparent that the relatively low mortality rate was not due to the specific action of any of the chemotherapeutic agents but were the result of the gradual decrease in the virulence of B. besnoiti following repeated serial passage in a foreign host, the rabbit.

/79.....

TABLE V.

TREATMENT DURING THE INCUBATION PERIOD, AND IN A LARGE NUMBER OF CASES ALSO DURING THE PERIOD OF REACTION.

Drug	Number treated	Severity of disease	Number that died
Anthiomaline	1	1( + +)	1
Aralen	2	2(+ +)	1
Formalin	4	4(+ +)	0
Mixtamycin	1	1(+ +)	1
Mixtamycin and Phenergan	1	1(+ +)	1
Mycostatin and Phenergan	1	1(+ +)	1
Pamaquin	1	1 (+ +)	0
Phenamidine	1	1 (+ +)	1
Pentamidine	2	2 (+ +)	0
Quinacrine	3	3 (+ +)	1
Spirotrypan	4	1 (+) 2(+ +) 1(+ + +)	0 1 1
Terramycin	2	1 (+) 1 (+ +)	0 1
Trypan blue	1	1 (+ +)	0
Tubomel (INH) and P.A.S.	2	2 (+ +)	0

In conclusion it can be stated that the apparent cures should be considered with full knowledge that spontaneous recovery in rabbits suffering from besnoitiosis is common. Consideration of these results permits the conclusion that no really satisfactory chemotherapeutic treatment has yet been found unless the curative effects of sulfamerazine should be fully confirmed.

The observations on the chemotherapy, and the criteria used for assessing the value of drugs as cures are presented with the hope that they will serve as guides for future investigations.

## PROPHYLAXIS

Consideration of the gradual dissemination of bovine besnoitiosis into the potential enzootic regions in the Northern Transvaal, and the sporadic outbreaks of equine besnoitiosis within the same regions, as well as in the Eastern Cape Province and Zululand (vide supra - Epizootology) makes it apparent that effective prophylactic measures for the prevention of the spread are dependent upon determining the natural mode of transmission and the host range of B. besnoiti in nature. Since the cyclical incidence during the summer months suggests that an arthropod may be involved in the transmission, the use of reliable arthropodocides seems to be a feasible control measure. As dipping tanks and spray races are available for the control of tick-borne diseases on many farms, the proposed prophylactic measure can, therefore, be conducted without extra costs. It may also be of value to eliminate chronically affected cattle as they may serve as reservoirs for the infection of vectors.

The development of a reliable method of immunization needs serious consideration as this will overcome defects of other prophylactic measures. The ideal method of immunization will involve the artificial infection of cattle, and controlling the ensuing reaction with a specific drug. Its application is dependent upon the availability of an adequate source of the infectious agent and a reliable chemotherapeutic agent. The former requirement can be fulfilled by using artificially infected rabbits as reservoirs, while the second one still needs to be determined.

Although experiments have shown that artificial infection of mature bulls is followed by a relatively mild form of besnoitiosis, observations on spermatogenesis have revealed that a temporary sterility may persist for periods of up to six months. Recognition of this complication makes it apparent that a specific remedy is urgently needed.

Another approach to the immunization is the artificial infection of very young stock. As severe reactions, following a natural infection, have not yet been observed

in this age group, it is believed that equally mild reactions will follow an artificial infection.

### IMMUNITY

Studies on the nature and duration of the immunity in bovine besnoitiosis were based on observations made on cattle that survived a natural infection, and on cattle, sheep, goats and rabbits which recovered from an artificial infection. Criteria used for assessing the immunity were founded on the persistence of parasitic cysts after recovery, and the clinical and spermatogenic response to challenge.

No reference to immunity could be found in the literature other than that of Hofmeyr (1945) who stated that farmers in the Rustenburg district (Transvaal) had never seen a second attack of besnoitiosis in cattle. The reason for the solid immunity became evident subsequently when it was found that recovered cattle remain carriers of parasitized cysts for life (vide supra - Pathogenicity). It may thus be concluded that naturally recovered cattle develop a premunity.

A few observations on the nature and duration of immunity following recovery from an artificial infection were conducted on an ox No. 5870 (Table B), a bull No. 5873, two billy goats Nos. 1952 and 3171 (Table G) and a ram No. 86569 (Table D of the Appendix). The immunity of ox No. 5870 was challenged 7 months after infection, that of bull No. 5873 after 13 and 36 months, that of goat No. 1952 after 6 and 19 months, that of goat No. 3171 after 4 and 18 months, and that of ram No. 86569 14 months after infection. In the case of the bull and the two billy goats attention was paid to the spermatogenesis after the first immunity test. Careful observations failed to reveal any clinical symptoms and indications of aspermatogenesis.

The immunity of 18 rabbits was challenged at various intervals of up to 112 days after recovery from an artificial infection. A thermal reaction which persisted for three days was observed in one rabbit two days after challenge. Examination of blood smears failed to reveal

any parasites. The remaining rabbits remained normal in every respect. Since sections prepared from skin biopsies taken at the time when the immunity tests were applied, revealed B. besnoiti cysts, it is concluded that the nature of the immunity in rabbits is the same as in cattle, namely an immunitas non sterilisans. It thus becomes apparent that the proposed method of immunization can be relied upon to impart a durable immunity.

#### SUMMARY AND CONCLUSIONS.

- (1) A brief historical review of bovine besnoitiosis is given.
- (2) The importance of the disease as a veterinary and economic problem is stressed.
- (3) The identification of the causal agent, Besnoitia besnoiti (Marotel, 1913) has made it possible to give a concise definition of the disease.
- (4) The synonyms of bovine besnoitiosis are listed.
- (5) The distribution of bovine and equine besnoitiosis in Europe and Africa, and the occurrence of murine besnoitiosis in the United States of America are recorded.
- (6) A detailed account of the aetiology, based on observations made on rabbits and cattle, is offered.
- (7) Systematic studies on the development of the parasite cyst revealed that the cyst wall is of somatic origin.
- (8) The inner and intermediate membranes of the wall are derived from the markedly hypertrophied polynucleated host cell, the histiocyte, while the outer wall is formed by collagenous fibres.
- (9) The term pseudocyst, as applied by Jacobs (1956) for the Toxoplasma cyst, where the cyst wall is also of somatic origin, should be used in the same sense for the Besnoitia cyst.
- (10) Certain chemical components of the parasite and cystic wall have been determined by histochemical tissue reactions.
- (11) Asexual reproduction by longitudinal binary fission within, either monocytes or histiocytes, is the only form of multiplication in the vertebrate host.

/83.....

- (12) The "Fuls" B. besnoiti strain has been maintained by serial passage in rabbits for 125 generations. A gradual decrease in virulence of the parasite was noticed as the passage level increased.
- (13) B. besnoiti remains viable in citrated blood for 48 hours at 20°C and for 96 hours at + 4°C.
- (14) A detailed survey of the complicated history of the nomenclature of certain Protozoa included in the genera Balbiana, Besnoitia, Eimeria, Fibrocystis, Gastrocystis, Globidium, Haplogastrocystis, Ileocystis, Lymphocystis, Sarcocystis and Toxoplasma is given.
- (15) Evidence has been brought forward that Besnoitia is a valid genus, that it is related to the genera Fibrocystis and Toxoplasma but distinct from any of the remaining genera.
- (16) Absence of any characteristic features does not permit placing the genera Toxoplasma, Besnoitia and Fibrocystis in any natural scheme of classification in the Protozoa, and hence it is proposed to group them as "Parasites of Doubtful Nature".
- (17) It has been determined that besides cattle and rabbits, sheep, goats and guinea pigs are susceptible, while two horses, a dog and several mice, rats, fowls and dassies failed to develop a microscopic infection and clinical symptoms after artificial infection.
- (18) The pathogenesis is discussed.
- (19) The natural mode of transmission is obscure.
- (20) Information on the epizootology is limited.
- (21) The symptomatology of bovine besnoitiosis in cattle, sheep, goats, rabbits and guinea pigs is described.
- (22) Bulls that survive a natural infection develop either a temporary or permanent sterility.
- (23) A temporary sterility, as determined by systematic microscopic semen examination of artificially infected animals, persisted for approximately six months in two bulls, for about three months in a billy goat, and four months in a ram.
- (24) Methods for making a diagnosis are described. In inapparent or atypical infections in male ruminants, aspermatogenesis can be used as a guide for making a diagnosis.
- (25) Investigations on bovine besnoitiosis have not advanced far enough to evolve reliable prophylactic measures.

- (26) Animals that survive either a natural or an artificial infection develop a durable premunity.
- (27) Photographs showing the course of the disease in a bull, the pronounced sclerodermatitis in a chronically affected animal, the development of a parasitic cyst in a bull and a rabbit, and the morphology and method of reproduction of B. besnoiti are presented.

#### ACKNOWLEDGMENTS.

In conclusion I wish to record my indebtedness to the Director of Veterinary Services, Union of South Africa, Dr. R.A. Alexander for granting facilities to conduct the investigations, and to my promotor, Prof. W.O. Neitz, who gave invaluable guidance, especially in the taxonomic studies and preparation of the article. Thanks are also due to Prof. H.P.A. de Boom, Mr. W. Gerneke and Mr. H.S. Walters for their assistance with the histology and histochemistry; Dr. H.O. Flanagan for his help in maintaining the strain: Mr. V.E. de Kock, Mr. C. Hall and Mr. F.J. van Pletzen for keeping records: Mr. A.M. de Bruyn for preparing the photographs: Mr. F. Boughton for his technical assistance, and Mrs. R. Archibald for her careful typing.

#### REFERENCES.

- ARAGAO, H. de B. (1911). Observacoes sobre algumas hemogregarine das aves. Mern. Inst. Oswaldo Cruz. Vol. 3, pp 54-64.
- ASHWORTH, J.H. (1923). On Rhinosporidium seeberi (Wernicke, 1903), with special reference to its sporulation and affinities. Trans. Roy. Soc. Edin. Vol. 13, p.301.
- BABUDIERI, B. (1931). Cited by Babudieri, B (1932). Sarcosporidi e le Sarcosporidiosi (Studio monografico). Arch. J. Protistenk. Vol. 76, pp. 423-580.
- BALBIANA, E.G. (1885). As cited by Wenyon (1926). Protozoology Vol. 2. Bailliere, Tindall and Cox, London.
- BARRAIRON, E. (1938). Contribution a le'étude étologie de l'anasarque du boeuf. Thesis, Toulouse Vet. Bull. Vol. 9, p.465.
- BENNETT, S.C.J. (1927). A peculiar equine Sarcosporidium in Anglo Egyptian Sudan. Vet. J. Vol. 83, p.297.

- BENNETT, S.C.J. (1928). Globidium infection. Ann. Rep. Sudan Vet. Serv. p.32.
- BENNETT, S.C.J. (1932). Globidium infection. Ann. Rep. Sudan Vet. Serv. pp. 38-41.
- BENNETT, S.C.J. (1933). Globidium infection in the Sudan. J. Comp. Path. Vol. 46, pp. 1-15.
- BENNETT, S.C.J. (1939). A case of so-called Globidium infection in a horse. Ann. Report Sudan Vet. Serv. p.37.
- BESNOIT, C. and ROBIN, V. (1912). Sarcosporidiose Cutanée chez une vache. Rec. Vet. Vol. 37, p. 649. Cited by NÖller (1920) and Wenyon (1926).
- BESNOIT, C. and ROBIN, V. (1913). Les reactions cellulaires dan la sarcosporidiose cutanée. Comp. Preud. Soc. Biol., Vol. 65, p.357.
- BESNOIT, C. and ROBIN, V. (1913). Sarcosporidiose cutanée une vache. Rec. méd. vét. Vol. 90, pp.327-328.
- BESNOIT, C. and ROBIN, V. (1914). Les lésions de la sarco-sporidiose cutanée des bovins dans leurs rapport avec l' histogénèse du tubercle. Rev. Vét. Vol. 71, p. 193.
- BIOCCA, E. (1953). Toxoplasma and Encephalitozoon. 6th Intern. Congr. Microbiol. Rome.
- BLANCHARD, R. (1885). Note sur les Sarcosporidies et sur un essai de classification de ces Sporozoaires. Bull. Soc. Zool. Paris. Vol. 10, P. 244.
- BOUGHTON, D.C. (1942). An overlooked macroscopic intestinal lesion of value in diagnosing bovine coccidiosis. North. Am. Vet. Vol. 23, pp. 173-175.
- BRUCE, E.A. (1921). Bovine coccidiosis in British Columbia with a description of the parasite Eimeria canadensis sp. n. J. Am. Vet. Med. Assoc. Vol. 58, pp. 638-662.
- BRUMPT, E. (1913). Précis de Parasitologie, 2nd ed. Paris, France.
- BRUMPT, E. (1915). As cited by Babudieri, B. (1932). Arch. f. Protistenk. Vol. 76, pp. 423-580.
- BRUMPT, E. (1927). Précis de Parasitologie, 4th ed. Paris, France.
- BRUMPT, E. (1949). Précis de Parasitologie 6th ed. Paris, France.
- BUGGE, G. (1910) Kokzidiose des Darmes bei Rindern. Deutsche Tierärztl. Wchnschr. Vol. 18. p. 461.

/86.....

- BÜTSCHLI, O. (1882). Protozoa Bronn's Kl. U. Ordn. des Tierreichs. Leipzig.
- CADEAC, (1884). Identité de l' éléphantiasis et de l' anasarque du boeuf. Description de cette maladie. Revue vétérinaire, 1884. p.521.
- CAMPBELL, J.G. (1954). Bangkok haemorrhagic diseases of chickens; an unusual condition associated with an organism of uncertain toxonomy. J. Path. Bacteriol. Vol. 68,(2) pp. 423-429.
- CARINI, A. (1909). Reproduction expérimentale de la Toxoplasmose du lapin. Bull. Soc. Path. exot. Vol. 2, p. 465 and 524.
- CHATTON, E. (1910). Le kyste de Gilruth. Arch. du Zool. exp. et generale. Vol. 45,(4) pp. 114-124.
- CHATTON, E. (1912). Review of paper by Bull and Gilruth. 1912. Bull. Inst. Pasteur. Vol. 10, p. 792.
- COLBACK, H. (1951). Personal communication. (Belgian Congo).
- COUTELEN, F. (1933). Sur la position systematique de Globidium mucosum (R. Blanchard, 1858), parasite du kangourou des rochers Macropus penicillatus. Ann. Paras. Num. comp. Vol.11, p.1.
- CROSS, J.B. (1947). A cytological study of Toxoplasma with special reference to its effect on the host's cell. J. Inf. Diseases. Vol. 80, pp.278-296.
- CUILLÉ, J. and CHELLE, P.L. (1937). Nouvelles recherches sur la transmission expérimentale de la maladie appelée "anasarque des bovides" (globidiose cutanée du boeuf). Bull. de l' Acad. de Méd. Vol. 118 pp. 217-219.
- CUILLÉ, J. and CHELLE, P.L. and BERLUREAU, F.(1936). Transmission expérimentale de la maladie dénommée "Sarcosporidiose cutanée du boeuf" (Besnoit et Robin) et déterminée par Globidium besnoiti. Bull. de l' Acad. de Méd. Vol. 115, pp. 161-163.
- DA CUNHA and TORRIS, (1926). As cited by Reichenow (1953). Lehrbuch der Protozoenkunde 6th ed. Gustav Fischer Verlag, Jena.
- DA CUNHA, A.M., and MUNIZ, J. (1928). Sur un nouveau sporozoaire, parasite du tatou. C.R. Soc. Biol. Vol. 98, p. 624.
- DARLING, S.T. (1910). Sarcosporidiosis in the Opossum and its experimental production in the guinea pig by intramuscular injection of Sporozoites. Bull. de la Soc. de Pathol. Exot. Vol.3, p.513.
- DE BOOM, H.P.A. (1947). Personal communication (Onderstepoort)
- DE KOCK, G. v.d. W. (1947). Personal communication (Onderstepoort).
- DE WET, G. (1950). Personal communication. (Pretoria).

- DOFLEIN, and REICHENOW, (1929). Lehrbuch der Protozoenkunde. Gustav Fischer Jena. 5 ed.
- D.V.S. Bechuanaland Protectorate (1957). Personal Communication.
- ENIGK, K. (1934). Zur Kenntniss des Globidium cameli und der Eimeria cameli. Arch. Protistenk. Vol. 83, pp. 371-380.
- FLESCHE, M. (1883). Über ein Sporozoon beim Pferde. Zool. Anzeiger. Vol. 6, pp. 396-397.
- FRANCO, E.E., and BORGES, I. (1916). Sur la sarcosporidiose Bovine. Arg. Inst. Bact. Camara Pestana. Vol. 4, pp. 269-289.
- FRENKEL, J.K. (1953a)<sup>d</sup>. Infections with organisms resembling Toxoplasma. Together with the description of a new organism: Besnoitia jellisoni. Estratto dagli Atti Del VI Congresso Internazionale di Microbiologica. Vol. 5, pp. 426-434.
- FRENKEL, J.K. (1953b). Infections with organisms resembling Toxoplasma. Riass. Comm. 6th Congr. Internat. Microbiol. Rome. Vol. 2, pp. 556-557.
- FRENKEL, J.K. (1955)<sup>e</sup>. Infections with organisms resembling Toxoplasma, together with the description of a new organism: Besnoitia jellisoni. Riass. Comm. 6th Congr. Internat. Microbiol. Rome. Vol. 5, pp. 426-434.
- FRENKEL, J.K. (1955). Ocular lesions in hamsters with chronic Toxoplasma and Besnoitia infection. Am. Jnl. Ophthalmol. Vol. 39, pp. 203-255.
- FRENKEL, J.K. (1956). Pathogenesis of Toxoplasmosis and of infections with organisms resembling Toxoplasma. Ann. of the N.Y. Acad. of Sc. Vol. 64, pp. 215-254
- FRENKEL, J.K. (1956) Effects of hormones on the adrenal necrosis produced by Besnoitia jellisoni in golden hamsters. J. Exptl. Med. Vol. 103, (3) pp. 375-398.
- FRENKEL, J.K. and FRIEDLANDER, S. (1951). Toxoplasmosis; Pathology of neonatal diseases, pathogenesis, diagnosis and treatment. Publ. Health Serv. No. 141 Washington D.C. 105 p. 91. ill.
- GARNHAM, P.C.C. (1950). Blood parasites of East African vertebrates, with a brief description of exoerythrocytic schizogony in Plasmodium pitmani. Parasitology. Vol. 40, pp. 328-337.
- GILRUTH, J.A. (1910). Notes on a protozoan parasite found in the mucus membranes of the abomasum of the sheep. Bull. Soc. Pathol. Exot. Vol. 3, pp. 297-298.
- GILRUTH, J.A. and BULL, L.B. (1912). Enteritis associated with infection of the intestinal wall of cyst-forming protozoa (Neosporidia) occurring in certain native animals. (Wallaby, Kangaroo and Wombat). Proceed. of the Roy. Soc. of Victoria.

d <sup>d</sup> Date 1953 - given for reprint.  
e <sup>e</sup> Date 1955- given for journal.

- GOING, J. (1927). Cited by Bennett S.C.J. (1933). Jnl. Comp. Path. and Therap. Vol. 46 pp. 1-15.
- GUYENOT, E.M., NAVILLE, A, and PONSE, K. (1922). Deux coccidies parasites de Tropidonotus natrix. Rev. Suisse Zool. Vol. 30, pp. 115-157.
- HADWEN, S. (1922). Cyst forming protozoa in reindeer and caribou and a Sarcosporidia of the seal. J. Am. Vet. Med. Ass. Vol. 61, p. 374.
- HAMMOND, D.M., BOWMAN, G.W., DAVIS, L.R., and SIMMS, B.T. (1946). The endogenous phases of the life cycle of Eimeria bovis. Jnl. Parasitol. Vol. 32, pp. 409-423.
- HARANT, H., and CAZAL, P. (1934). Remarques sur le genre Globidium : Globidium navillei n. sp. parasite de la couleuvre. Ann. Parasitol. Vol. 12, pp. 162-169.
- HASSAN, S.R. (1935). Globidiosis in Indian cattle, with a description of a new species. Indian J. Vet. Sc. Anim. Husband. Vol. 5, pp. 177-183.
- HENMERT-HALSWICK, A. (1943). Infektion mit Globidium leuckarti beim Pferd. Ztschr. Veterinärk. Vol. 55, pp. 192-199.
- HENNING, M.W. (1956). Animal Diseases in South Africa. 3rd. ed. Central News Agency Ltd., South Africa.
- HENRY, A. (1913). Le travail de M.M. Besnoit et Robin. Egalement communiqué a la Société des Sciences Vétérinaires de Lyon (Séance du 17 Novembre 1912). Rec. med. vet. Vol. 90(9) p. 328.
- HENRY, A, and MASSON, G. (1922). Cited by Neveu-Lemaire, M. (1943). Traite de protozoologie médicale et vétérinaire. Vigot Frères, Editeurs, Paris.
- HENRY, A, and MASSON, G. (1932). Considerations sur le genre Globidium. Globidium cameli n. sp. parasite du dromadaire. Ann. Parasitol. Vol. 10, pp. 382-401.
- HERIN, V.V. (1952). Note sur le existence de la Globidiose bovine an Ruanda-Urundi. Ann. Soc. Belgo Med. Trop. Vol. 32(2) pp. 155-160.
- HERTER, L. (1950). Personal communication. (Potgietersrus, Transvaal).
- HIREGAUDAR, L.S. (1956). A record of Globidium leuckarti (Flesch) from a horse in India. Current Science. Vol. 25, pp. 334-335.
- HOBMAIER, M. (1922). Globidium infektion beim Fohlen. Berl. Tierärztl. Wochschr. Jg. 1922, p.100.
- HOFMEYR, C.F.B. (1945). Globidiosis in cattle. J. South Afric. Vet. Med. Ass. Vol. 16, pp. 102-109.
- JACOBS, Leon. (1956). Propagation, Morphology and Biology of Toxoplasma. Ann. of the N.Y. Acad. of Sc. Vol. 64, pp. 154-179.

- JELLISON, W.L. (1953). Cited by Frenkel (1953). Estratto dadi Atti. Del. VI. Congresso Internazionale di Microbiologie. Vol. 5, pp. 426-434.
- JELLISON, W.L. (1956). On the nomenclature of Besnoitia besnoiti a protozoan parasite. Ann. New York Acad. of Sc. Vol. 64, pp. 268-270.
- JELLISON, W.L. (1956). Transmission of the protozoan Besnoitia jellisoni by ingestion. Ann. of the N.Y. Acad. of Sc. Vol. 64, pp. 271-274.
- JOHNSON, W.T. (1930). Director's biennial report, 1928-1930. Ore. Agric. Expl. Station.
- KÜHN, J. (1865). Untersuchungen über die Trichinenkrankheit de Schweine. Inst. d. Univ. Halle, p. 68.
- KUPKE, A. (1923). Untersuchungen über Globidium leuckarti Flesch. Inaugural Dissertation. Tierärztliche Hochschule, Berlin.
- KUPKE, A. (1923). Untersuchungen über Globidium leuckarti Flesch. Zeitschr. Infektionshr. d. Haust. Vol. 24, p. 210.
- LAINSON, R. (1958). Atoxoplasma Garnham, 1950, in an English sparrow (Passer domesticus domesticus Linn.). Trans. Roy. Soc. Trop. Med. Hygiene. Vol. 52, pp. 15-16.
- LAINSON, R. (1958). Observations on the development and nature of pseudocysts and cysts of Toxoplasma gondii. Transact of the Royal Soc. of Trop. Med. and Hygiene. Vol. 52(5), pp. 396-407.
- LAINSON, R. (1959). A note on the deviation of Toxoplasma infection in the guinea pig. Ann. Trop. Med. and Parasit. Vol. 53,(1), pp. 27-34.
- LAIRD, M. (1959). Atoxoplasma paddae (Aragao) from several South Pacific silver eyes (Zosteropidae) and a New Zealand rail. Jnl. Parasit. Vol. 45, pp. 47-52.
- LAMPRECHT, M.C. (1950). Personal communication. (Vryheid, Natal).
- LANKESTER, Ray (1926). Cited by Wenyon (1926). Protozoology Vol. 2. Bailliére, Tindall and Cox, London.
- LEITAO, J.L. da Silva (1949). Globidiose bovina per Globidium besnoiti. Rev. de Med. Vet. (Portugal). Vol. 44 (330), pp. 152-156.
- LEUCKART, R. (1879). Die Parasiten des Menschen. 2de ed. I. Leipzig.
- LILLIE, R.D. (1948). Histopathological Technic. Blakerton Co., Philadelphia.
- LOUW, F. (1954). Personal communication. (Rustenburg, Transvaal)
- MANSVELT, R.P. (1953). Personal Communication. (Northern Transvaal).

- MANWELL, R.D. and DROBECK, H.P. (1951). Mammalian toxoplasmosis in birds. Exper. Parasitol. Vol. 1, p. 83.
- MAROTEL, G. (1907). Un nouveau sporozoaire intestinal chez le veau. Bull. Soc. Sc. Vet. Lyon. Vol. 10, pp. 249-254.
- MAROTEL, M. (1912). Discussion of paper by Besnoit and Robin. Bull. et Mém. de la Société des Sciences Veterinaires de Lyon et de la Société de Médecine Vétérinaire de Lyon et du Sud-Est. Vol. 15, pp. 196-217.
- MARULLAZ, M. (1913). An sujet d' un *Toxoplasma* des oiseaux. Bull. Soc. Path. exot. Vol. 6, pp. 323-326.
- MASKE, (1893). Gregarine im habmagen des Schafes. Zeitschr. f. Fleisch und Milk hygiene. Vol. 4, pp.28-29.
- MEESER, M.F.N. (1954). Personal communication. (Lydenburg, Transvaal).
- MIESCHER, F. (1843). Ueber eigenthümliche Schläuche in den Muskeln einer Hausmans. Ber. u.d. Verhandl. Naturf. Ges. Basel. Vol. 5, p. 198.
- MOUSSU, G. and MAROTEL, G. (1902). La coccidiose du mouton et son parasite. Arch. Parasitol. Vol. 6, pp.82-98.
- MÜLLER, H. (1914). Über das Vorkommen von Coccidien und Schizonten bei gesunden Rindern. Inaug. Diss. Leipzig. Kiel. 47 pp.
- NEVILLE, A. (1931). Les sporozoaires. Mém. Soc. Phys. Hist. Nat. Genève. Vol. 21, pp. 1-223.
- NAVILLE, A. (1936). A propos du *Globidium (Gastrocystis) cameli* (Henry et MASSON). Ref. Fac. Sc. Univ. Istanbul. Vol. 1, 11 1-9.
- NEAVE, S.A. (1939). Nomenclator Zoologicus. Vol. 1, p. 423.
- NEVEU-BEMAIRE, M.(1943). Traite de Protozoologie Médicale et Vétérinaire. Vigot Frères, Editeurs, Paris.
- NICOLLE, C., and MANCEAUX, L.(1909). Sur un protozoaire nouveau du gondi. C.R. Acad. Sci. Vol.147, p.763.
- NÖLLER, W. (1920). *Globidium (Gastrocystis, Besnoitia) Prowazek.*s Handbuc der Pathogenen Protozoen. Leipzig. Vol. 2, pp. 919-993.
- PILLERS, A.W.N. (1928). *Globidium smithi* in the mucosa of the small intestine of a bull. Vet. Jnl. Vol. 84, pp. 462-464.
- POLS, J.W. (1952). Unpublished observations. (Onderstepoort).
- POLS, J.W. (1954a). The artificial transmission of *Globidium besnoiti* (Marotel 1912) to cattle and rabbits. Jnl. South African Med. Ass. Vol. 25(2), pp.37-44.

- POLS, J.W. (1954b). Preliminary notes on the behaviour of Globidium besnoiti (Marotel 1912), in the rabbit. Jnl. South African Vet. Med. Asso. Vol. 25(3), pp. 45-48.
- RAILLIER, A. (1919). La coccidiose intestinale on dysentric coccidienne des bovines. Réc. Méd. Vet. Vol. 95, pp. 5-27.
- REICHENOW, E. (1929). Lehrbuch der Protozoenkunde. 5th ed. Gustav Fischer, Jena.
- REICHENOW, E. (1940). Ueber das Kokzid der Equiden Globidium leuckarti. Ztschr. Infektionskr. der Haustiere. Vol. 56, pp. 126-134.
- REICHENOW, E. (1953). Lehrbuch der Protozoenkunde 6th ed. Gustav Fischer Verlag, Jena.
- REICHENOW, E. and CARINI, A. (1937). Über Eimeria travassosi und die Gattung Globidium. Arch. Protistenk. Vol. 88, pp. 374-386.
- RIEDMÜLLER, L. (1928). Ueber die Morphologie, Uebertragungsversuche und klinische Bedeutung des Sporadischen Abortus des Rindes vorkommenden Trichomonaden. Centralbl. Bakt. 1. Abt. Vol. 108, pp. 103-118.
- RIVOLTA, S. (1878). Della gregarinosi dei polli e dell'ordinaments delle gregarine e dei psorospermi degli animali domestici. Giorn. Anat. Fisiol. e Pat. Anim. Pisa. 1877-78, Vol. 10, p.220.
- SABIN, A.B., and FELDMAN, H.A. (1948). Dyes as microchemical indication of a new immunity phenomenon affecting a protozoan parasite (Toxoplasma). Science. Vol. 180 (2815), pp. 660-663.
- SCHNEIDER, A. (1875). Contributions à l'histoire des grégarines des invertébrés de Paris et de Roscoff. Arch. Zool. Vol. 4, p. 493.
- SCHULZ K.C.A., and THORBURN, J.A. (1955). Globidiosis, a cause of dermatitis in the horse. Jnl. S.African Vet. Med. Assoc. Vol. 26, pp. 39-43.
- SCOTT, J.W. (1930). The sarcosporidia; a critical review. J. Parasit. Vol. 16, pp. 111-130.
- SMITH, T. (1893). Preliminary notes on a sporozoon in the intestinal villi of cattle. Bull. U.S. Dept. Agr., Bur. An. Ind. Vol. 3, pp. 73-78.
- SPIEGL, A. (1925). Ein bisher nicht unbekanntes Coccid beim Schaf. Zeitschr. Infekskr. Haust. Vol. 22, pp. 265-269.
- SPINDLER, L.A. (1947). A note on the fungoid nature of Sarcocystis. Proc. Helm. Soc. Wash. Vol. 11(16), pp. 28-30.
- SPINDLER, and ZIMMERMAN. (1945). The biological status of Sarcocystis, U.S. Bur. of An. Ind. J. Parasit. Vol. 31, (Supl.13).

- THOMAS, A.D. (1943). Personal communication. (Onderstepoort).
- THOMAS, A.D., FREAN, J. and NREITZ, W.O. (1943). Personal communication. (Onderstepoort).
- THOMAS, A.D., and ADELAAR T.B. (1947). Personal communication. (Onderstepoort).
- THOMAS, A.D. (1950). Personal communication. (Pretoria).
- TRIFFIT, M.J. (1926). Some sporezoan parasites found in the intestinal wall of Bennett's wallaby (Macropus bennetti). Protozoology, No. 11, p. 31.
- TRIFFIT, M.J. (1927). A note on the occurrence of a Sarcocyst Parasitic in a wallaby. Protozoology. No. 3, April, 1927, pp. 75-76.
- TRIFFIT, M.J. (1928). Further observations on the development of G. gilruthi. Protozoology. No. 4 Sept. 1928, pp. 83-90.
- VAN DER MERWE, J.P. (1953). Personal communication. (Pietersburg, Transvaal).
- VAN DER VIVER, B. (1945) Personal communication. (Pretoria).
- VAN NEDERVEEN, H.J. (1922). Gastrocystis smithi (Railliet) in den Darmwand van het Rind. Tydschr. Microb. Geneesk. Vol. 8, pp. 121-128.
- VAN THIEL, P.H. (1956). The taxonomic status of Toxoplasma gondii. J. Microbiol. and Serol. Vol. 22, No. 3, pp. 248-256.
- VAN THIEL, P.H. and LAARMAN, J.J. (1956). Therapy and transmission of experimental Toxoplasmosis. 6th Congr. Intern. Microbiol. Rome. Vol. 5, pp. 445-448.
- VOGES, O. (1901). Das Mal de Cadéras der Pferde in Südamerika. Berlin. Tierärztl. Wochschr. Jg. 17, p. 598.
- WHYON, G.M. (1926), Protozoology. Vol. 2. Bailliére, Tindall and Cox, London.
- WHYON, G.M. and SCOTT, H.H. (1925). Demonstration of sections of intestines of Bennett's wallaby. Trans. Roy. Soc. Trop. Med. and Hyg. Vol. 14, p. 7.
- WESTPHAL, A. (1953). Die Toxoplasmen als Trypanosomidae; 6th Congr. Internat. Microbiol. Rome. Vol. 2, pp. 379-381.

/93.....

- WILLIAMS, H.B. (1933). Cited by Bennett, S.C.J.(1933).  
Jnl. Comp. Path. and Therap. Vol. 46, pp. 1-15.
- YAKIMOFF, W.L. and GALOUZO, J.G. (1927). Zur Frage der  
Rindercoccidien. Arch. J. Protisk. Vol. 58,  
pp. 185-200.
- YAKIMOFF, W.L., and GALOUZO, J.G. (1927). Die Darm-  
coccidiose der Rinder in Russland. Centralbl.  
Bakt. etc. 1 Orig. Vol. 103, pp. 97-108.
- ZOOLOGICAL RECORD, (1916). Vol. 53, p.23.
- ZUBLIN, E. (1908). Beitrag zur kenntuis der roten Ruhr.  
des Rindes (Dysenteria coccidiosa bovis). C.R.  
Soc. Biol. Vol. 50, p.123.

- A. The course of severe bovine besnoitiosis in a naturally infected bull No. 6851 (Fig. 1-6).

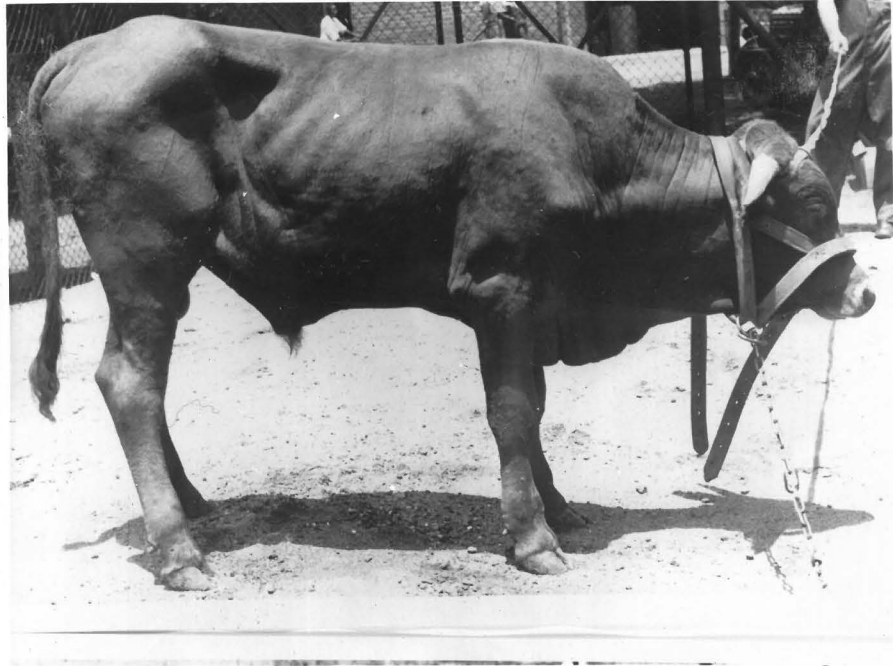


Fig. (1) Clinical symptoms during the first phase of the disease. Note the moderate oedema of the limbs.



Fig. (2) Hindview of bull on same day.



Fig. (3) Clinical symptoms during second phase of the disease, thirteen days later. Note decubitus wound on hip and well defined thick skin folds.



Fig. (4) Hindview of bull on same day.



Fig. (5) Clinical symptoms during third phase of the disease, 35 days later. Note pronounced emaciation, markedly thickened skin folds and circumscribed areas of alopecia.



Fig. (6) Hindview of bull on same day.

- B. Advanced case of chronic bovine besnoitiosis in a naturally infected cow. No. 6547 (Fig. 7 and 8).

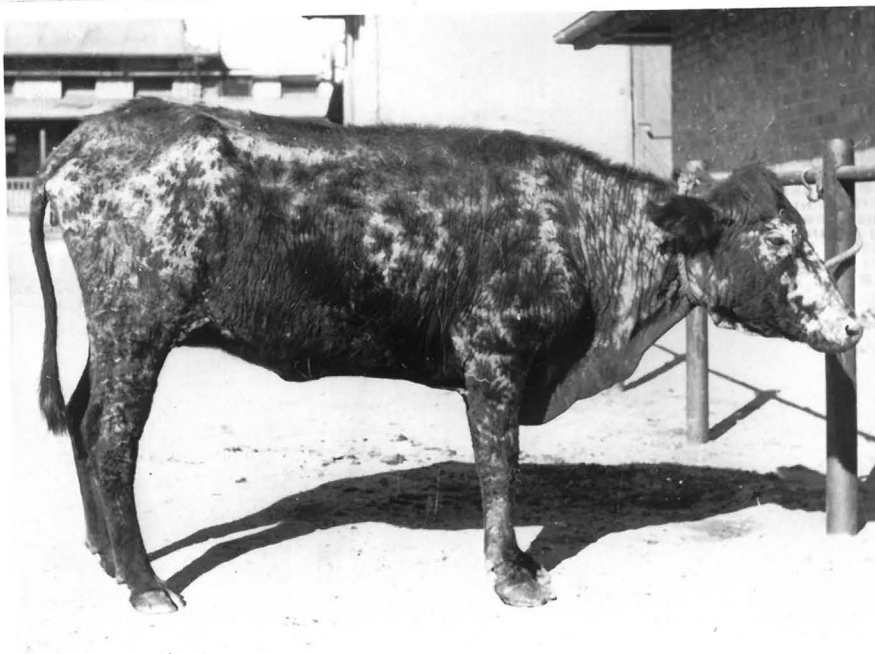


Fig. (7) Clinical symptoms four months after infection. Note emaciation extensive alopecia and indurated wrinkly skin.



Fig. (8) Head of cow on same day. Note dry and crusty nasal discharge and alopecia.

- C. Typical case of bovine besnoitiosis in an artificially infected rabbit No. 89 (Fig. 9 and 10).

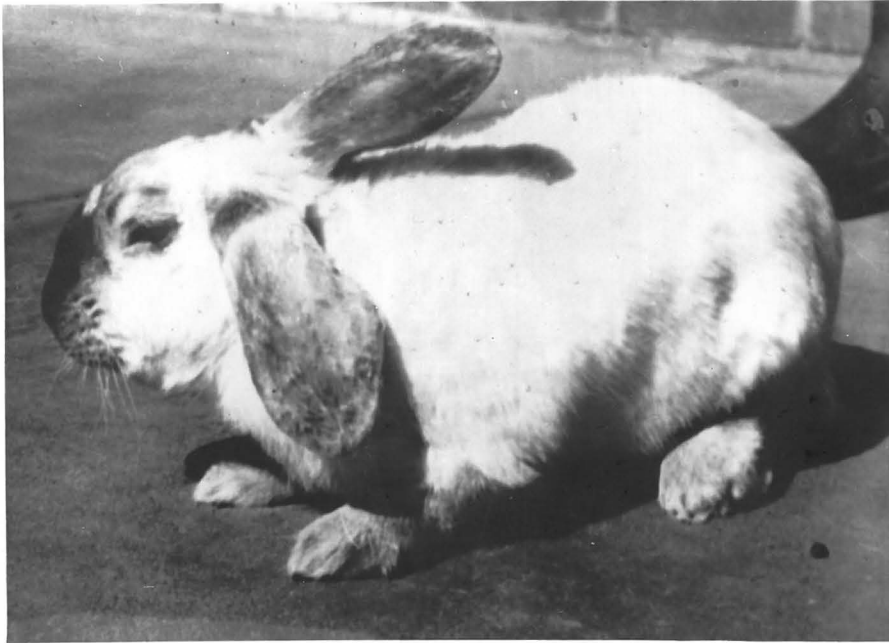


Fig. (9) Pronounced oedematous swelling of ears particularly the left one.

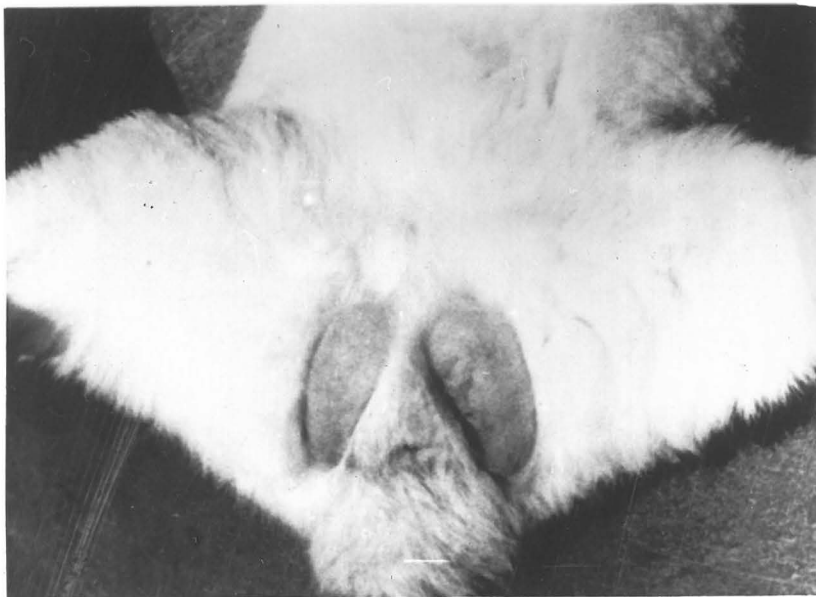


Fig. (10) Same rabbit showing oedematous swelling of scrotum.

- D. Skin sections of bull 6851 showing the development of B besnoiti cysts over a period of five weeks (Fig. 11-16).

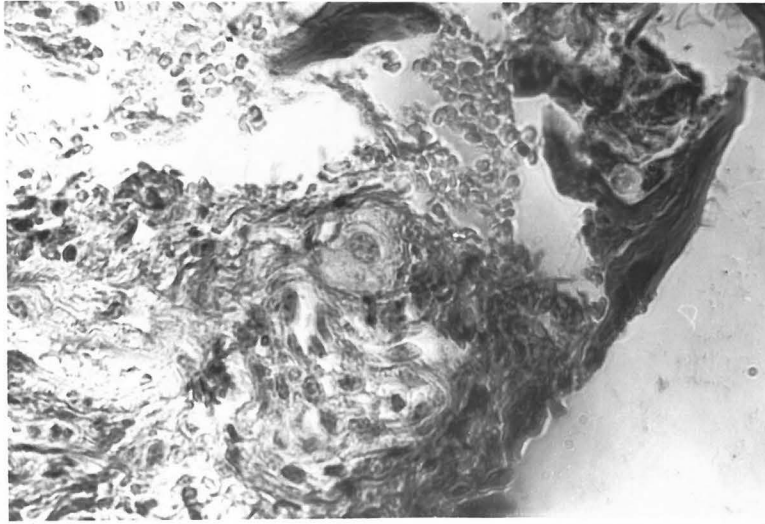


Fig. (11) Enlarged uninuclear histiocyte surrounded by connective tissue fibres four days after height of thermal reaction x 480.

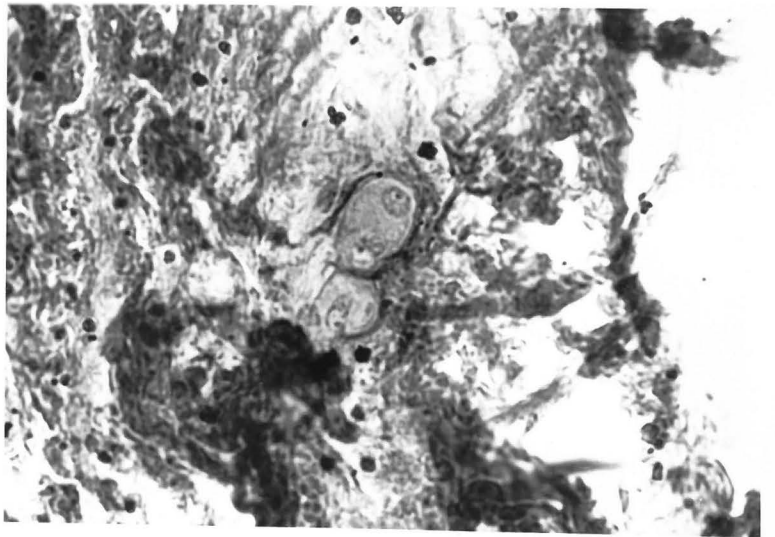


Fig. (12) Enlarged uninuclear histiocyte containing two B besnoiti parasites on the 7th day x480.

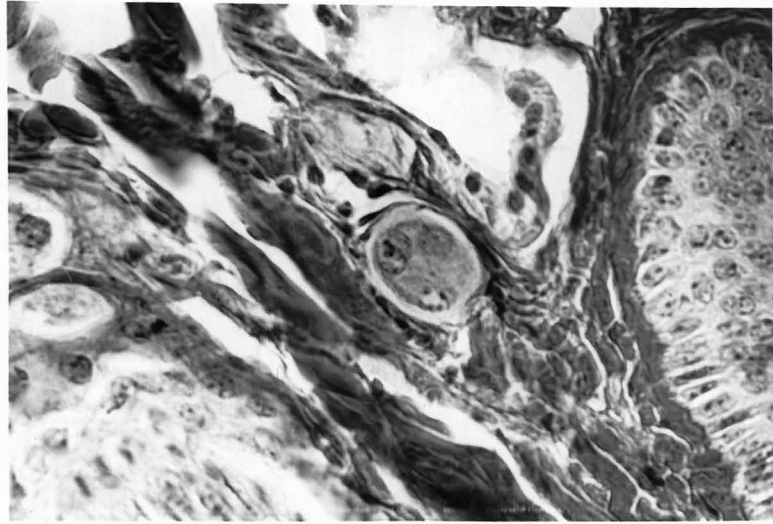


Fig.(13) Enlarged binucleated histiocyte containing four parasites on 8th day x480.



Fig. (14) Group of multinucleated histiocytes containing vacuoles with parasites and surrounded by connective tissue fibres on 13th day x480.

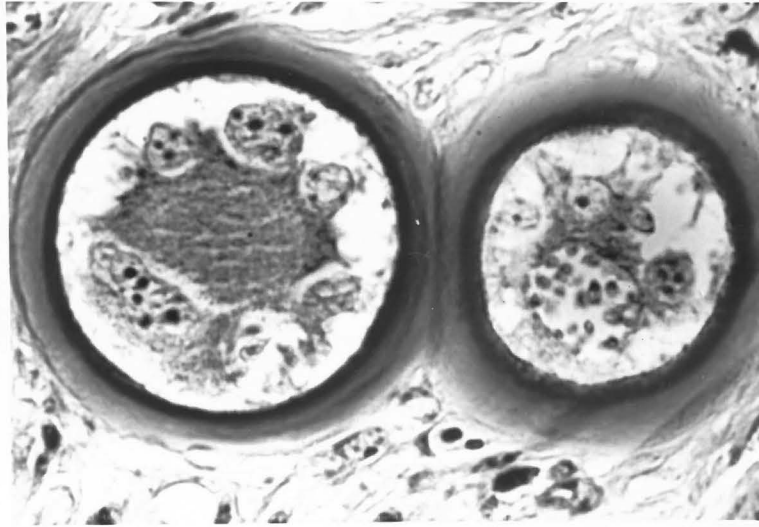


Fig.(15) Young cysts with well-defined walls each containing several hostcell nuclei, and vacuoles harbouring parasites on 24th day x480.

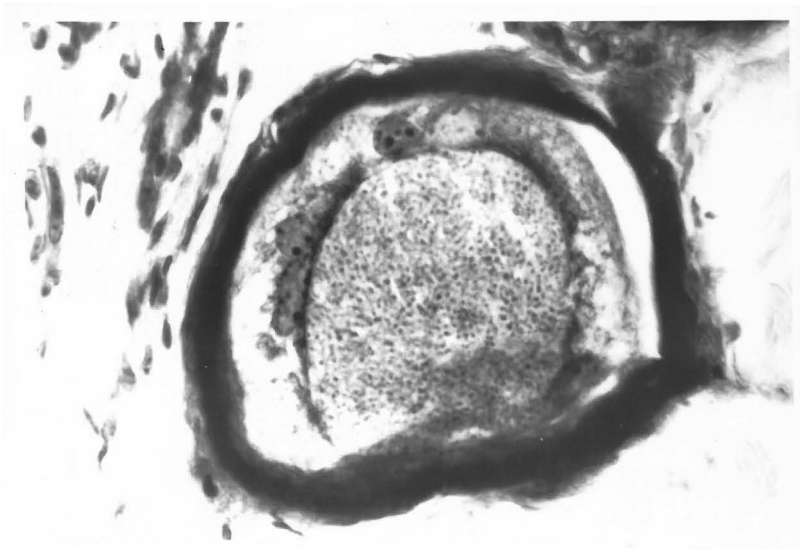


Fig.(16) Immature cyst with distinct wall and inner membrane surrounded by distorted multi-nucleated histiocyte on 35th day x480.

E. Skin sections of cow No. 6547 prepared four months after natural infection. (Fig. 17 and 18).

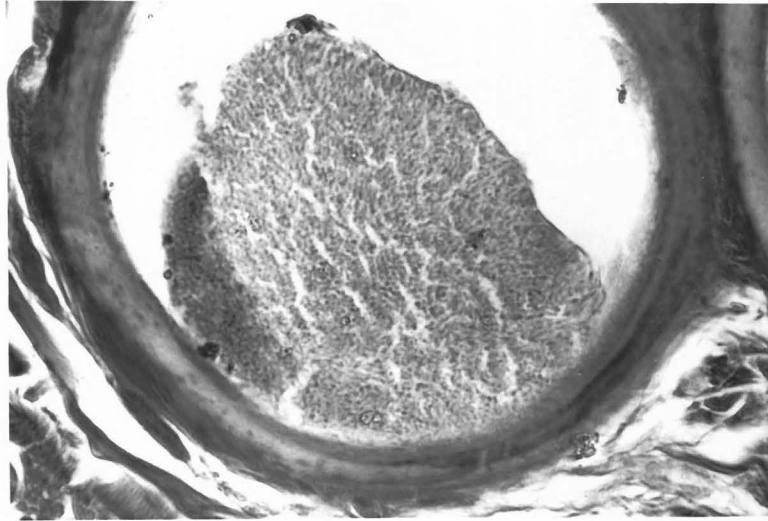


Fig.(17) Mature cyst showing well developed wall and numerous parasites x480.

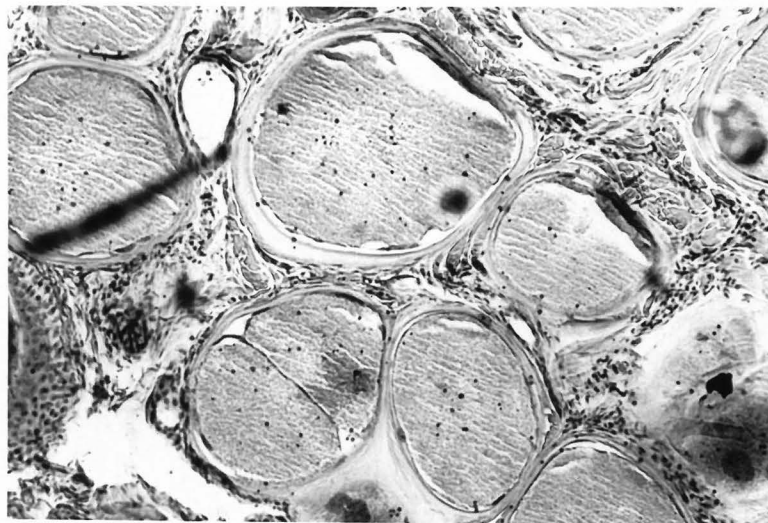


Fig.(18) Group of mature cysts one showing bilobular parasitic masses x120.

- F. Skin sections of an artificially infected rabbit No. 89 prepared 14 and 18 days after height of thermal reaction. (Fig. 19 and 20).

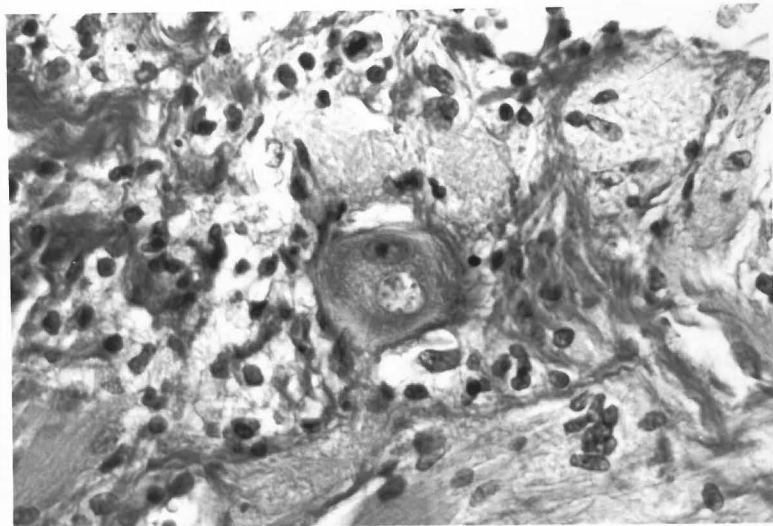


Fig.(19) Uninucleated histiocyte harbouring a vacuole with parasites x 480.

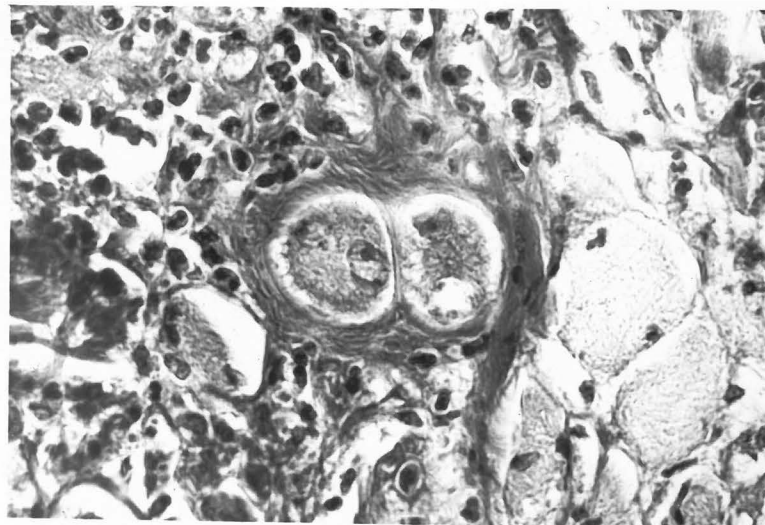


Fig.(20) Two developing cysts harbouring a host cell nucleus, and vacuoles with parasites and surrounded by connective tissue fibres x480.

G. Parasites as seen in blood smears of different rabbits. (Fig. 21-24).

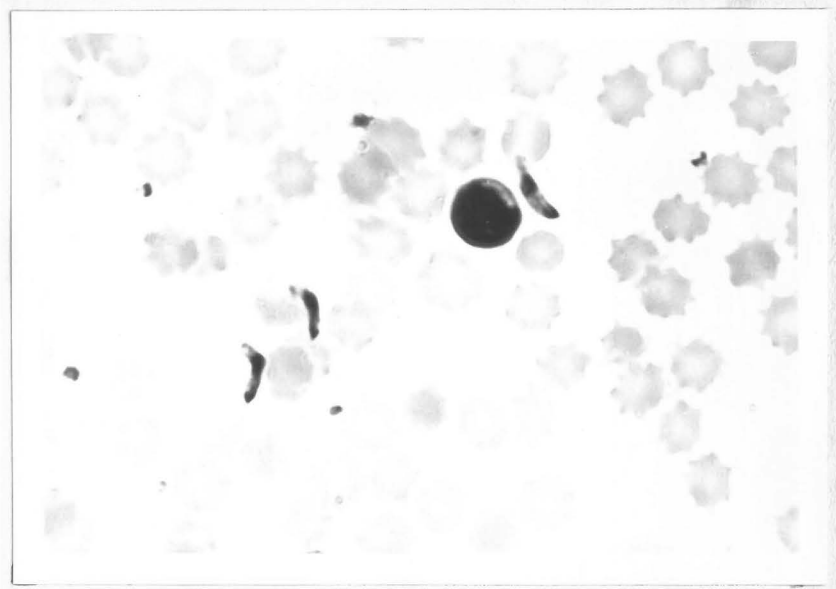


Fig.(21) Contracted extracellular parasites x1125.

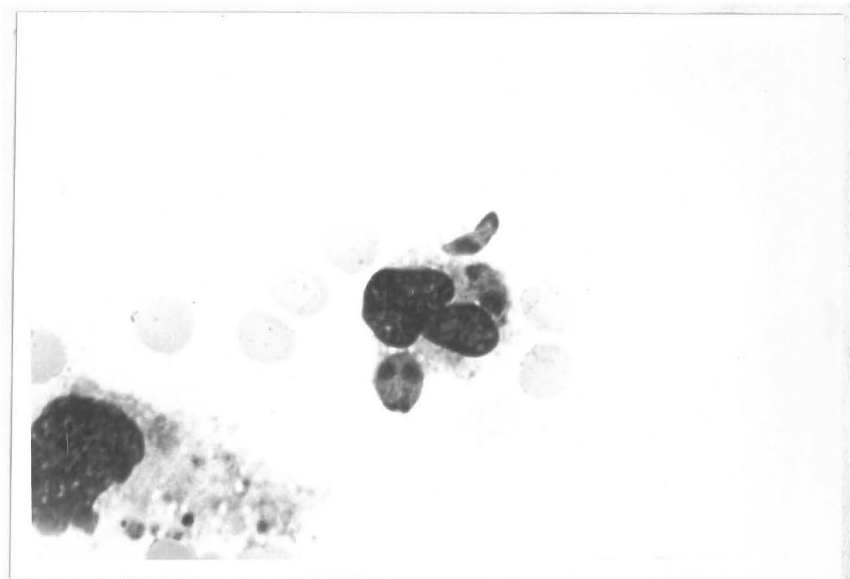


Fig.(22) Monocyte with distorted cytoplasm and surrounded by parasites, one of which, is binucleated and in the process of binary division. Note the darkly staining cap-like structure at polar end of the uniuucleated free parasite x1125.

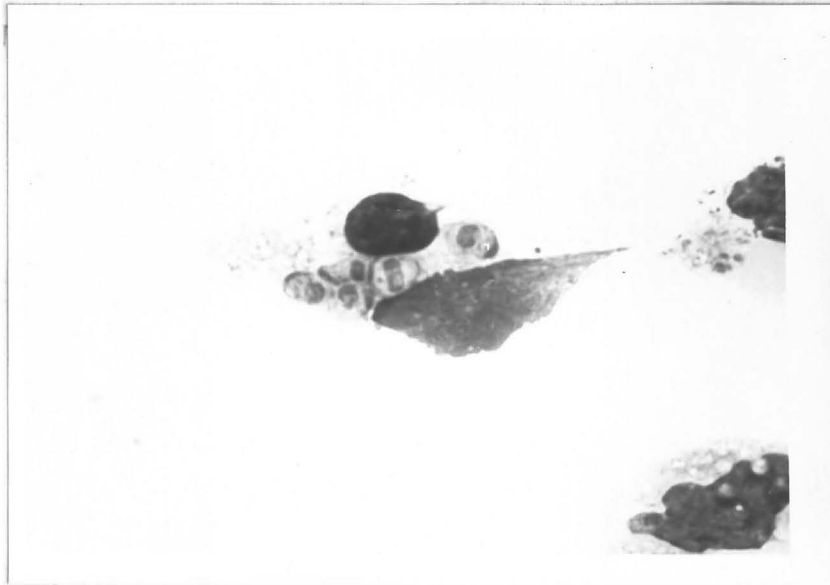


Fig.(23) Monocyte with distorted cytoplasm and parasites in the process of reproduction. Note nuclear division in centrally situated parasite x 1125.

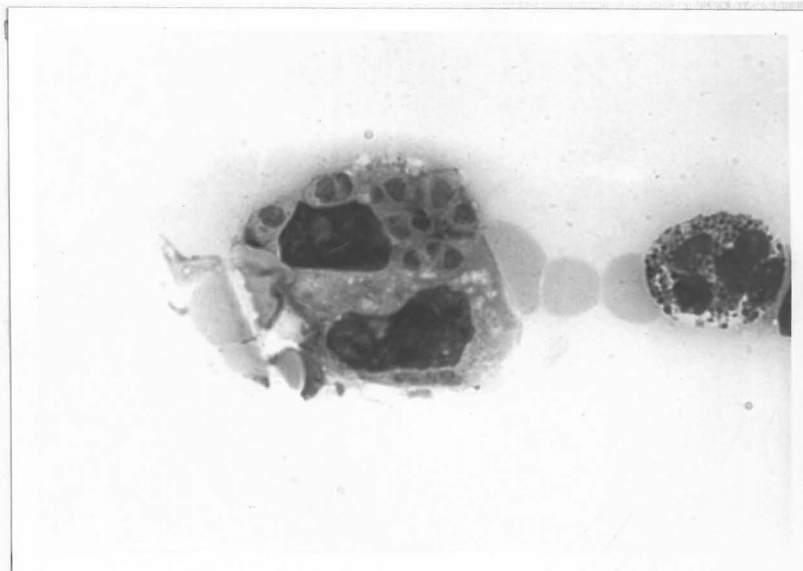


Fig.(24) Monocyte harbouring parasites. Note schizont-like structure which probably represents an aberrant form. x1125.

APPENDIX TABLE A

RESISTANCE OF B. besnoiti TO PHYSICAL AND CHEMICAL AGENTS

Rabbit No.	Donor No.	Date of infection	Dose of blood in ml.	Stored blood		Incubation period in days	Examination of blood smears			Clinical Symptoms	Duration of disease in days	Severity of reaction	Result	
				Time	Max. temp. °C.		Anticoagulant	Date	Para-sites					Oedema
344	338	6/10/54	4.0 ip.	0	39.0	Sodium citrate	6	18/10/54	+	+	104.4	9	++	Died
								19/10/54	+					
								20/10/54	++					
								21/10/54	++					
346	338	7/10/54	5.0 ip.	0.5 hour	20.0	Potassium oxalate	7	20/10/54	++	+	105.4	8	++	Recovered.
								21/10/54	+++					
347	338	7/10/54	5.0 ip.	0	39.0	<sup>x</sup> Edington's solution	15	2/11/54	+	+	106.4	7	++	Recovered.
								3/11/54	++					
348	338	7/10/54	5.0 ip.	0	39.0	<sup>x</sup> Edington's solution	10	21/10/54	+++	+++	105.4	5	+++	Died.
								22/10/54	+++					
1261	348	22/10/54	5.0 ip.	1.5 hours	20.0	<sup>x</sup> Edington's solution	-	-	-	-	103.0	-	-	No reaction.
1262	348	22/10/54	5.0 ip.	1.5 hours	20.0	<sup>x</sup> Edington's solution	-	-	-	-	103.8	-	-	No reaction.
1259	348	22/10/54	5.0 ip.	1.5 hours	20.0	Sodium citrate	10	3/11/54	+	+	106.0	8	++	Recovered.
1260	348	22/10/54	5.0 ip.	1.5 hours	20.0	Sodium citrate	10	3/11/54	++	+	106.8	7	++	Recovered.
								4/11/54	+					
								5/11/54	+					
1257	344	22/10/54	5.0 ip.	24 hours	20.0	Sodium citrate	5	1/11/54	+	+	105.8	8	++	Redovered.
1258	344	22/10/54	5.0 ip.	24 hours	20.0	Sodium citrate	10	3/11/54	++	+	105.6	7	++	Recovered.
								4/11/54	++					
								5/11/54	+++					
1267	344	2/11/54	4.0 ip.	12 days	-76.0	Sodium citrate	-	-	-	-	103.0	-	-	No reaction.
	1279	<sup>3</sup> 1/12/54	5.0 ip.	0	39.0	Sodium citrate	9	14/12/54	+	-	106.6	7	++	Recovered.
								15/12/54	++					
1268	344	2/11/54	4.0 ip.	12 days	-76.0	Sodium citrate	-	-	-	-	103.2	-	-	No reaction.
1748	346	5/1/57	4.0 ip.	26 months	-76.0	Sodium citrate	-	-	-	-	102.6	-	-	No reaction.
1752	346	5/1/57	4.0 ip.	26 months	-76.0	Sodium citrate	-	-	-	-	100.4	-	-	No reaction.
1749	119	5/1/57	4.0 ip.	30 months	-76.0	Sodium citrate	-	-	-	-	103.0	-	-	No reaction.
1296	1289	20/12/54	5.0 ip.	0	39.0	Sodium citrate	9	3/1/55	++	-	106.0	9	++	Recovered.
								4/1/55	+++					
1314	1296	6/1/55	5.0 ip.	48 hours	20.0	Sodium citrate	12	24/1/55	+	+	106.2	7	++	Recovered.
								25/1/55	++					
1316	1296	6/1/55	5.0 ip.	48 hours	20.0	Sodium citrate and antibiotics	-	-	-	-	103.0	-	-	No reaction.
	1341	<sup>3</sup> 4/3/55	5.0 ip.	0	39.0	Sodium citrate	5	10/3/55	+	+	104.8	9	+	Died
1317	1296	6/1/55	5.0 ip.	48 hours	20.0	Sodium citrate and antibiotics	-	-	-	-	106.6	-	-	No reaction.
	1341	<sup>3</sup> 4/3/55	5.0 ip.	0	39.0	Sodium citrate	7	16/3/55	+++	-	105.4	8 7	++	Died.
								17/3/55	++					

APPENDIX TABLE A (continued).

RESISTANCE OF *B. besnoiti* TO PHYSICAL AND CHEMICAL AGENTS.

Rabbit No.	Donor No.	Date of Infection	Dose of blood in ml.	Stored blood		Incubation period in days	Examination of blood smears		Clinical Symptoms		Duration of disease in days	Severity of reaction	Result	
				Time	Max. temp. °C.		Anticoagulant	Date	Para-sites	Oedema				Max. temp. °F.
1307	1294	30/12/54	5.0 ip	0	39.0	Sodium citrate	5	10/1/55	+++	+	104.8	7	++	Recovered.
1319	1307	13/1/55	5.0 ip	72 hours	+4.0	Sodium citrate	7	24/1/55 25/1/55	++ +++	+	105.8	4	++	Died.
1320	1307	14/1/55	5.0 ip	72 hours	+4.0	Sodium citrate and ∅ antibiotics	8	24/1/55 25/1/55	+ +++	+	106.2	6	++	Died.
1322	1307	14/1/55	5.0 ip	96 hours	+4.0	Sodium citrate	6	25/1/55 26/1/55	++ +++	-	105.4	7	++	Died.
1323	1307	14/1/55	5.0 ip	96 hours	+4.0	Sodium citrate and ∅ antibiotics	-	-	-	-	103.4	-	-	No reaction.
	1341	*4/3/55	5.0 ip	0	39.0	Sodium citrate	10	16/3/55	++	-	105.6	9	++	Recovered.
1750	1307	5/1/57	4.0 ip	24 months	-76.0	Sodium citrate	-	-	-	-	103.0	-	-	No reaction.
	1815	*19/3/57	5.0 ip	0	39.0	Sodium citrate	8	31/3/59	+	+	105.6	7	++	Recovered
1482	1474	28/1/56	5.0 ip	0.5 hours	20.0	Heparin	4	2/2/56 3/2/56	+ +	+	105.6	6	++	Died.
1486	1474	29/1/56	5.0 ip	24 hours	37.0	Sodium citrate	-	-	-	-	103.2	-	-	No reaction.
1489	1474	31/1/56	5.0 ip	96 hours	20.0	Sodium citrate	-	-	-	-	104.0	-	-	No reaction.
1535	1510	27/4/56	5.0 ip	30 days	-18.0	Sodium citrate	-	-	-	-	103.2	-	-	No reaction.
	1552	*14/6/56	5.0 ip	0	39.0	Sodium citrate	5	?	?	-	104.6	7	+	Recovered.
1536	1510	27/4/56	5.0 ip	30 days	-18.0	Sodium citrate	-	-	-	-	104.0	-	-	No reaction.
	1552	*14/6/56	5.0 ip	0	39.0	Sodium citrate	6	25/6/56	++	+	105.8	8	++	Recovered.
1803	1791	22/2/57	5.0 ip	0	39.0	Sodium citrate and ∅ antibiotics	14	14/3/57	+	-	106.4	10	++	Recovered.
1804	1791	22/2/57	10.0 ip	0	39.0	Sodium citrate and ∅ antibiotics	8	6/3/57	+	-	105.4	6	++	Died

\*Immunity, xEdington's solution = 500 ml. water  
500 ml. glycerine  
5.0 ml. carbolic acid  
5.0 gm. potassium oxalate.

∅ Antibiotics = 500 units of penicillin and  
150 micrograms streptomycin  
per 1.0 ml. blood.

APPENDIX TABLE B.

ARTIFICIAL TRANSMISSION OF B. BESNOITI TO CATTLE

Animal No.	Origin	Donor	Date of infection	Dose of blood ml. i.v.	incubation period in days	Microscopical observations				Clinical symptoms	Remarks
						Trophozoites in smears		Cysts in skin section			
						Date	Results	Date	Results		
Cow 6793	Lydenburg Received 31/1/53	Naturally infected ox which died from besnoitiosis.	28/12/53	1000	?	-	-	20/1/54	+	No fever or oedema of subcutaneous tissues.	Very mild case.
Ox 6840	Rustenburg Received 29/1/54 Natural case.	-	-	-	-	1/2/54	Gland +	18/2/54	+	Fever 103 <sup>40</sup> F 28/1/54 Oedematous swelling of front limbs below the knees and dewlap.	Relatively mild symptoms. Skin after 2 months showing scleroderma accompanied by depilation.
Heifer 6839	Rustenburg Received 29/1/54	6840	28/1/54	1000	10	8/2/54	Blood + Gland +	20/2/54	+	Febrile reaction for 3 days. Highest temperature 106.8°F. Oedematous swelling below the knees from 12/2/54 to 16/2/54.	Mild reaction showing slight scleroderma and depilation.
Bull 6851	Pretoria district	-	-	-	-	4/2/54	Blood + Gland +	9/2/54	3 +	Febrile reaction for 3 days. Highest temperature 106.6°F. Oedematous swelling of all limbs, dewlap and skin of thorax from 2/2/54 to 6/2/54.	Acute reaction accompanied by anasarca and scleroderma. Died on 14/3/54 in a very cachectic condition.
Ox 5895	Available animal	6851	2/2/54	1000	6	-	-	8/3/54	+	Febrile reaction for 3 days. Highest temperature 106°F. No swellings.	Very mild case.
Ox 5887	Available animal	(R) R.T.	15/2/54	13	7	-	-	10/3/54	+	No swellings noticed. Highest temperature 107°F.	Mild reaction.
Ox 5870	Available animal.	Ox 5887	25/2/54	250	9	6/3/54	Blood +			Oedema of front limbs. Highest temperature 105.8°F.	

+ = Parasites very rare. 2 + = Parasites fairly frequently. (R) = Rabbit.

APPENDIX TABLE C

Serial Passage of the Fuls B. Besnoiti Strain in Rabbits.

Gen	Donor	No. of recipients	Date of infection	Dose of blood in ml.	No. that reacted	Serial No. of rabbit selected	History of selected donor								
							Incub. period in days	Examination of blood smears	Clinical symptoms	Duration of disease in days	Severity of reaction.	Died	Killed		
								Date	Parasites	Oedema	Highest Temperature °F				
1	Ox 6840	1	28/1/54	5.0 ip.	1	RB	3	10/2/54	+	++	-	3	++	+	-
2	RB	3	10/2/54	3.0 iv.	3	2	6	16/2/54 17/2/54	+	++	106.2	12	++	+	-
3	2	2	18/2/54	10.0 sc.	2	24	14	4/3/54 5/3/54 6/3/54	++	++	106.8	3	+++	+	-
4	24	2	4/3/54	2.5 sc	2	39	13	18/3/54 19/3/54 20/3/54	+	+	107.0	3	++	+	-
5	39	2	19/3/54	2.5 ip.	2	54	8	29/3/54 31/3/54 1/4/54	+	+	106.2	4	++	+	-
6	54	6	31/3/54	2.0 ip.	5	58	6	7/4/54	++	+	106.6	4	++	+	-
7	58	2	7/4/54	2.0 ip.	2	67	6	15/4/54 20/4/54	+	++	106.4	5	++	+	-
8	67	6	20/4/54	3.0 ip.	4	80	6	29/4/54 30/4/54 1/5/54	+	+	105.6	5	++	+	-
9	80	4	29/4/54	2.5 ip.	4	83	7	10/5/54	+	-	105.6	-	+	-	+
10	83	6	10/5/54	2.5 ip.	5	96 98	8 9	19/5/54 19/5/54 20/5/54	+	-	105.8 105.2	-	+	-	+
11	96 & 98	3	19/5/54	2.5 ip.	2	106	13	5/6/54 8/6/54 10/6/54	+	+	106.0	38	+	+	-
12	106	2	5/6/54	4.0 ip.	1	109	7	15/6/54 16/6/54 17/6/54 18/6/54	+	++	105.6	12	++	+	-

APPENDIX TABLE C (continued)

Serial Passage of the Fuls *B. besnoiti* Strain in Rabbits

Gen	Donor	No. of recipients	Date of infection	Dose of blood in ml	In: ip.	No. re-acted	Serial No. of rabbit selected	Incub. period in days	History of selected donor				Died	Killed		
									Examination of blood smears	Clinical symptoms	Duration of disease in days	Severity of reaction				
								Date	Parasites	Oedema	Highest Temperature OF					
13	109	2	12/6/54	4.0	ip.	2	112	9	28/6/54	+	-	105.8	7	++	+	-
			15/6/54	2.0	ip.											
			15/6/54	4.0	ip.		114	7	28/6/54	++	-	105.6	6	++	+	-
14	112 & 114	4	28/6/54	5.0	ip.	2	115	7	3/7/54	+	++	106.4	8	++	+	-
									5/7/54	+						
15	115	2	5/7/54	5.0	ip.	2	119	5	14/7/54	++	+	106.2	5	++	+	-
16	119	1	14/7/54	6.0	ip.	1	304	5	26/7/54	++	+	106.0	3	++	+	-
17	304	2	26/7/54	5.0	ip.	2	305	6	-	-	++	106.6	5	++	+	-
							306	8	-	-	+	105.6	6	+	+	-
18	305 & 306	2	17/8/54	10.0	ip.	2	316	12	31/8/54	+	-	104.8	5	+	+	-
19	316	3	31/8/54	5.0	ip.	2	320	13	13/9/54	+	+	105.2	4	++	+	-
20	320	3	11/9/54	4.0	ip.	3	326	9	23/9/54	++	+	106.0	9	++	+	-
21	326	3	23/9/54	4.0	ip.	2	333	8	5/10/54	++	++	105.8	4	++	+	-
							334	7	6/10/54	+	+	105.0	15	+	+	-
22	333 & 334	3	5/10/54	5.0	ip.	3	339	8	16/10/54	++	++	105.2	6	+++	+	-
									18/10/54	++						
									19/10/54	+++						
23	339	4	16/10/54	4.0	ip.	4	1255	6	26/10/54	++	+++	106.0	7	+++	+	-
									27/10/54	++						
									28/10/54	+++						
									29/10/54	+++						
24	1255	4	27/10/54	5.0	ip.	4	1264	6	6/11/54	+	+	106.6	6	++	+	-
									8/11/54	++						
25	1264	5	8/11/54	5.0	ip.	3	1273	8	19/11/54	+	+	105.6	6	++	+	-
									20/11/54	+++						
26	1273	5	19/11/54	5.0	ip.	3	1279	7	27/11/54	+	+	106.6	5	++	+	-
									29/11/54	++						
									1/12/54	+++						

APPENDIX TABLE C (Cont.)

Serial Passage of the Fuls *B. besnoiti* Strain in Rabbits.

Gen	Donor	No. of recipients	Date of infection	Dose of blood in ml.	No. that reacted	Serial No. of rabbit selected as donor	Incu. period in days	History of selected donor.							
								Examination of blood smears		Clinical symptoms		Duration of disease in days	Severity of reaction.	Died	Killed
Date	Parasite	Oedema	Highest temperature of												
27	1279	4	29/11/54	5.0 ip.	4	1286	7	7/12/54	+	+	105.4	4	+++	+	-
								8/12/54	++						
								9/12/54	++						
								10/12/54	+++						
28	1286	4	10/12/54	5.0 ip.	4	1291	4	17/12/54	++	-	104.6	6	++	+	-
								18/12/54	++						
29	1291	4	18/12/54	5.0 ip.	4	1294	6	28/12/54	+++	-	105.8	8	++	+	-
								30/12/54	+						
30	1294	4	30/12/54	5.0 ip.	4	1305	8	7/1/55	+	+	105.6	2	++	+	-
								8/1/55	++						
31	1305	4	8/1/55	5.0 ip.	4	1311	6	18/1/55	+	+	105.6	9	++	+	-
								20/1/55	++						
								21/1/55	+						
32	1311	3	20/1/55	5.0 ip.	3	1327	10	31/1/55	++	-	106.2	3	++	+	-
								1/2/55	+++						
33	1327	4	1/2/55	5.0 ip.	4	1338	8	11/2/55	++	-	104.2	8	++	+	-
								12/2/55	+						
34	1338	3	11/2/55	5.0 ip.	2	1344	8	25/2/55	++	-	106.2	?	++	-	+
35	1344	4	25/2/55	5.0 ip.	4	1349	6	5/3/55	+	+	106.0	?	++	-	+
36	1349	3	7/3/55	5.0 ip.	3	1353	6	17/3/55	++	+	106.4	6	++	+	-
								18/3/55	+						
37	1353	3	17/3/55	5.0 ip.	3	1356	5	26/3/55	++	+	106.4	8	++	+	-
								28/3/55	+						
								29/3/55	+						
38	1356	3	26/3/55	5.0 ip.	3	1360	8	4/4/55	+	-	106.6	3	+++	+	-
								7/4/55	+++						
39	1360	3	7/4/55	4.0 ip.	3	1361	4	15/4/55	+	+	105.6	6	++	+	-
								16/4/55	++						

APPENDIX TABLE C (cont.)

Serial Passage of the Fuls B. besnoiti Strain in Rabbits.

Gen	Donor	No. of recipients	Date of infection	Dose of blood in ml.	No. that reacted	Serial No. of rabbit selected as donor	History of selected donor.									
							Incub. period in days	Examination of blood smears	Clinical symptoms			Duration of disease in days.	Severity of reaction	Died	Killed	
							Date	Parasites	Oedema	Highest temperature of						
40	1361	3	16/4/55	4.0 ip.	3	1365	7	25/4/55	+	-	105.0	3	++	+	-	
								26/4/55	++							
								27/4/55	++							
41	1365	4	26/4/55	4.0 ip.	4	1369	6	5/5/55	+	+	105.6	8	++	+	-	
								6/5/55	+							
42	1369	4	6/5/55	5.0 ip.	4	1376	9	20/5/55	+	-	105.8	13	+	+	-	
43	1376	4	20/5/55	5.0 ip.	3	1379	8	30/5/55	+	+	105.4	4	++	+	-	
44	1379	4	30/5/55	5.0 ip.	3	1383	5	9/6/55	+	+	107.0	?	+	-	+	
45	1383	4	10/6/55	5.0 ip.	4	1388	13	28/6/55	+	+	105.8	6	++	+	-	
46	1388	4	28/6/55	5.0 ip.	2	1389	5	5/7/55	+	+	105.4	10	++	+	-	
47	1389	4	6/7/55	5.0 ip.	2	1394	7	18/7/55	+	+	105.8	5	++	+	-	
48	1394	4	18/7/55	5.0 ip.	2	1397	9	30/7/55	+	+	105.8	5	++	+	-	
49	1397	4	1/8/55	5.0 ip.	2	1401	9	-	-	-	105.0	8	+	+	-	
				5.0 ip.		1403	10	9/8/55	+	-	105.0	5	++	+	-	
50	1401 & 1403	4	12/8/55	5.0 ip.	3	1406	8	22/8/55	+	+	107.0	5	++	+	-	
								23/8/55	++							
51	1406	4	23/8/55	5.0 ip.	4	1410	8	6/9/55	+	-	106.6	10	++	+	-	
52	1410	4	6/9/55	5.0 ip.	1	1414	7	16/9/55	+	-	107.0	6	++	+	-	
53	1414	4	16/9/55	5.0 ip.	3	1417	7	26/9/55	+	+	105.2	4	++	+	-	
54	1417	4	27/9/55	5.0 ip.	3	1424	10	8/10/55	+	-	105.6	6	++	+	-	
55	1424	4	8/10/55	5.0 ip.	4	1427	8	17/10/55	+	+	104.2	3	++	+	-	
								18/10/55	++							
56	1427	4	18/10/55	5.0 ip.	4	1431	10	29/10/55	+	-	106.0	4	++	+	-	
57	1431	4	29/10/55	5.0 ip.	3	1435	10	10/11/55	++	-	105.8	4	++	+	-	
58	1435	4	10/11/55	5.0 ip.	3	1439	7	19/11/55	++	-	106.4	?	++	-	+	
59	1439	4	19/11/55	5.0 ip.	4	1441	6	28/11/55	++	-	106.8	6	+++	+	-	

APPENDIX TABLE C (cont.)

Serial Passage of the Fulls B. besnoiti Strain in Rabbits.

Gen.	Donor	No. of recipients	Date of infection	Dose of blood in ml	No. that reacted	Serial No. of rabbit selected as donor	Incu. period in days	History of selected donor.							
								Examination of blood smears		Clinical symptoms		Duration of disease in days	Severity of reactors	Died	Killed
								Date	Parasite	Oedema	Highest temperature °F				
60	144A	4	29/11/55	5.0 ip.	4	1446	6	5/12/55	+	+	106.6	6	+++	+	-
								6/12/55	+						
								7/12/55	++						
								8/12/55	+++						
61	1446	4	6/12/55	5.0 ip.	4	1451	7	14/12/55	+	-	107.8	?	++	-	+
								15/12/55	++						
62	1451	4	15/12/55	5.0 ip.	4	1453	7	23/12/55	++	+	105.6	3	++	+	-
63	1453	4	24/12/55	3.0 ip.	4	1459	6	3/1/56	++++	+	106.6	?	++	-	+
64	1459	4	3/1/56	3.0 ip.	4	1468	9	16/1/56	+	+	105.0	?	++	-	+
65	1468	4	16/1/56	5.0 ip.	2	1475	7	24/1/56	+	-	106.0	?	++	-	+
66	1475	4	26/1/56	5.0 ip.	2	1478	7	4/2/56	++	-	105.2	?	++	-	+
67	1478	4	4/2/56	5.0 ip.	4	1493	7	15/2/56	++	+	105.0	?	++	-	+
68	1493	4	15/2/56	5.0 ip.	3	1503	10	1/3/56	+++	-	105.6	?	++	-	+
69	1503	3	1/3/56	5.0 ip.	2	1509	12	16/3/56	+	+	104.4	?	++	-	+
70	1509	2	16/3/56	5.0 ip.	2	1514	6	23/3/56	+	-	105.8	4	++	+	
								24/3/56	++						
71	1514	4	23/3/56	5.0 ip.	4	1517	7	4/4/56	+	+	105.6	?	++	-	+
72	1517	4	5/4/56	5.0 ip.	4	1523	7	16/4/56	+	++	106.2	?	++	-	+
								17/4/56	+++						
73	1523	4	16/4/56	5.0 ip.	4	1527	8	28/4/56	+	-	104.8	?	++	-	+
								2/5/56	+						
74	1527	3	28/4/56	5.0 ip.	2	1537	15	18/5/56	+	+	105.0	?	++	-	+
75	1537	2	19/5/56	5.0 ip.	1	1556	8	30/5/56	+	-	105.8	?	++	-	+
76	1556	4	30/5/56	5.0 ip.	4	1563	9	15/6/56	+	-	105.6	?	++	-	+
77	1563	4	15/6/56	5.0 ip.	4	1581	6	29/6/56	++	+	106.2	?	++	-	+
78	1581	3	29/6/56	5.0 ip.	3	1590	8	12/7/56	+	++	107.6	4	+++	+	-
79	1590	4	12/7/56	5.0 ip.	4	1597	5	24/7/56	++	+	105.2	3	++	+	-

APPENDIX TABLE C (cont.)  
Serial Passage of the Fuls B. besnoiti Strain in Rabbits.

Gen	Donor	No. of recipients	Date of infection	Dose of blood in ml.	Inoculation	No. of rabbits re-acted	Serial No. of selected donor	History of selected donor									
								Incub. period in days	Examination of blood smears		Clinical symptoms			Duration of disease in days	Severity of reaction	Died	Killed
									Date	Parasite	Oedema	Highest temperature of					
80	1597	4	24/7/56	5.0	ip.	4	1607	7	7/8/56	+	-	104.0	?	+	-	+	
							1608	8	8/8/56	+	-	104.6	?	+	-	+	
81	1607 & 1608	4	8/8/56	5.0	ip.	2	1614	7	20/8/56	+++	-	105.0	?	++	-	+	
82	1614	1	20/8/56	5.0	ip.	1	1633	7	31/8/56	++	+	106.0	4	+++	+	-	
83	1633	2	31/8/56	5.0	ip.	2	1641	5	14/9/56	++	-	105.0	?	++	-	+	
84	1641	3	14/9/56	5.0	ip.	3	1650	6	26/9/56	++	-	105.8	?	++	-	+	
85	1650	2	26/9/56	5.0	ip.	2	1668	5	8/10/56	+++	++	105.4	3	+++	+	-	
86	1668	4	8/10/56	5.0	ip.	4	1681	5	16/10/56	+++	-	105.4	?	++	+	-	
87	1681	4	16/10/56	5.0	ip.	3	1688	6	27/10/56	+	+	106.0	?	+	-	+	
88	1688	4	27/10/56	5.0	ip.	4	1695	8	9/11/56	++	-	104.4	?	+	-	+	
89	1695	4	9/11/56	5.0	ip.	2	1704	8	21/11/56	++	+	106.2	?	++	-	+	
90	1704	4	21/11/56	5.0	ip.	2	1716	8	29/11/56	++	+	106.0	3	++	+	-	
91	1716	4	29/11/56	5.0	ip.	4	1722	7	10/12/56	+	-	104.4	?	+	-	+	
92	1722	4	10/12/56	5.0	ip.	3	1730	7	20/12/56	+	-	105.8	?	+	-	+	
93	1730	4	20/12/56	5.0	ip.	3	1737	6	2/1/57	+++	-	104.8	?	++	-	+	
94	1737	3	2/1/57	5.0	ip.	1	1745	10	21/1/57	++	-	104.4	?	+	-	+	
95	1745	2	21/1/57	5.0	ip.	2	1763	8	4/2/57 5/2/57	++ ++	+	105.2	3	++	+	-	
96	1763	4	4/2/57	5.0	ip.	1	1777	4	14/2/57	+++	-	105.0	?	++	-	+	
97	1777	4	14/2/57	5.0	ip.	1	1791	3	22/2/57	+	-	105.8	?	++	-	+	
98	1791	2	22/2/57	5.0	ip.	2	1801	4	5/3/57	++	+	105.6	?	++	-	+	
99	1801	4	5/3/57	5.0	ip.	3	1813	6	15/3/57	+	-	106.8	?	++	-	+	
100	1813	4	15/3/57	5.0	ip.	2	1821	7	26/3/57	++	-	105.4	?	++	-	+	
101	1821	4	26/3/57	5.0	ip.	2	1830	9	8/4/57	++	-	103.6	?	++	-	+	
102	1830	4	8/4/57	5.0	ip.	3	1836	8	23/4/57	++	++	108.0	10	+++	+	-	
103	1836	4	23/4/57	5.0	ip.	2	1839	5	3/5/57	+	+	104.6	?	++	-	+	
104	1839	4	3/5/57	5.0	ip.	4	1847	6	13/5/57	++	-	105.50	?	++	-	+	
105	1847	4	13/5/57	5.0	ip.	3	1852	7	24/5/57	+	-	105.4	?	++	-	+	

APPENDIX TABLE C (cont.)

Serial Passage of the Fuls *B. besnoiti* Strain in Rabbits.

Gen	Donor	No. of recipients	Date of infection	Dose of Blood in ml.	No. re-acted	Serial No. of rabbit selected as donor	Incub. period in days	Examination of blood smears.		Clinical Symptoms			Duration of disease in days.	Severity of reaction.	Died	Killed
								Date	Parasite	Oedema	Highest temperature of					
:106:	1852	: 4	:24/5/57	:5.0 ip.:	: 3	: 1857	: 7	:4/6/57	:+ +	: -	:106.0	: ?	:+ +	: -	: +	
:107:	1857	: 4	:4/6/57	:5.0 ip.:	: 4	: 1861	: 7	:13/6/57	:+	: -	:105.4	: ?	:+ +	: -	: +	
:108:	1861	: 4	:13/6/57	:5.0 ip.:	: 3	: 1865	: 5	:25/6/57	:+ +	: -	:105.8	: ?	:+ +	: -	: +	
:109:	1865	: 4	:25/6/57	:5.0 ip.:	: 3	: 1870	: 6	:10/7/57	:+	: -	:105.0	: ?	:+ +	: -	: +	
:110:	1870	: 3	:10/7/57	:5.0 ip.:	: 1	: 1879	: 4	:17/7/57	:+ + +	: -	:106.4	: 9	:+ +	: +	: -	
:111:	1879	: 4	:17/7/57	:5.0 ip.:	: 4	: 1884	: 7	:29/7/57	:+	: -	:105.4	: ?	:+ +	: -	: +	
:112:	1884	: 2	:29/7/57	:5.0 ip.:	: 2	: 1891	: 6	:8/8/57	:+	: +	:105.8	: ?	:+ +	: -	: +	
:113:	1891	: 2	:8/8/57	:5.0 ip.:	: 2	: 1902	: 5	:21/8/57	:+ +	: -	:105.2	: ?	:+ +	: -	: +	
:114:	1902	: 4	:21/8/57	:5.0 ip.:	: 4	: 1908	: 6	:31/8/57	:+	: +	:105.8	: ?	:+ +	: -	: +	
:115:	1908	: 4	:31/8/57	:5.0 ip.:	: 3	: 1917	: 7	:11/9/57	:+	: -	:106.4	: ?	:+ +	: -	: +	
:116:	1917	: 4	:11/9/57	:5.0 ip.:	: 4	: 1921	: 5	:20/9/57	:+	: -	:106.0	: ?	:+ +	: -	: +	
:117:	1921	: 4	:20/9/57	:5.0 ip.:	: 4	: 1924	: 7	:1/10/57	:+	: -	:106.2	: ?	:+ +	: -	: +	
:118:	1924	: 4	:1/10/57	:5.0 ip.:	: 1	: 1933	: 6	:12/10/57	:+	: -	:105.8	: ?	:+ +	: -	: +	
:119:	1933	: 4	:12/10/57	:5.0 ip.:	: 3	: 1939	: 5	:23/10/57	:+	: -	:106.0	: ?	:+ +	: -	: +	
:120:	1939	: 2	:23/10/57	:5.0 ip.:	: 1	: 1944	: 6	:2/11/57	:+ +	: +	:106.2	: ?	:+ +	: -	: +	
:121:	1944	: 2	:2/11/57	:5.0 ip.:	: 1	: 1948	: 6	:13/11/57	:+ +	: -	:105.4	: 10	:+ +	: +	: -	
:122:	1948	: 4	:13/11/57	:5.0 ip.:	: 2	: 1953	: 7	:26/11/57	:+	: -	:105.4	: ?	:+ +	: -	: +	
:123:	1953	: 2	:27/11/57	:5.0 ip.:	: 1	: 1961	: 3	:4/12/57	:+	: -	:104.8	: ?	:+	: -	: +	
:124:	1961	: 4	:4/12/57	:5.0 ip.:	: 2	: 1965	: 9	:17/12/57	:+	: -	:105.4	: ?	:+ +	: -	: +	
:125:	1965	: 4	:17/12/57	:5.0 ip.:	: 2	: 1970	: 7	:30/12/57	:+ + +	: -	:105.2	: ?	:+ +	: -	: +	

## APPENDIX TABLE D.

## OBSERVATIONS ON ARTIFICIALLY INFECTED GOATS AND SHEEP.

Gen.	Animal No.	Donor	Date of infection	Blood iv.	Incuba- tion pe- riod in days	Examina- tion of blood smears	Clinical symptoms		Remarks	Biological test on rabbits	
							Oedema	Highest temperature °F		Rabbit No.	Severity of re- action.
1	G.79328	R 327 (Gen.20)	28/9/54	35.0 ml.	8	-	++	107.0	Alopecia in anterior half of body. Observed for 6 months and recovered. Cysts seen in skin sections.	R 1251 R1252	++ ++
1	G.88537	R 1468 (Gen.64)	16/1/56	30.0 ml.	5	-	-	106.2	Observed for three-and-a-half months and killed. Cysts seen in skin sections.	R 1476 R 1484	++ ++
2	G.88549	G.88537	25/1/56	50.0 ml.	4	-	-	106.6	Died of acute peritonitis a month later. No cysts seen in skin sections.	R 1491	++
3	G.88536	G.88549	3/2/56	50.0 ml.	7	-	-	106.8	Died from haemorrhagic enteritis two-and-a-half months later. No cysts seen in skin sections.	R1499 R1500	++ ++
1	Sh.86569	R 327 (Gen.20)	28/9/54	35.0 ml.	7	-	-	107.0	Was challenged 28/11/56 (Donor R 1441, Gen 59). No reaction noted. Killed 2/5/56. No cysts seen in skin sections.	R 349 R 350	++ ++
1	Sh.89632	R 1468 (Gen.64)	16/1/56	30.0 ml.	5	-	-	107.2	Observed for three-and-a-half months. Aborted 8 weeks after highest temperature. Killed 2/5/56. No cysts in skin sections.		
2	Sh.615	Sh.89632	25/1/56	30.0 ml.	6	-	-	106.8	Observed for three-and-a-half months. Killed 2/5/56. No cysts in skin sections.	R1492	++
3	Sh.145	Sh.615	3/2/56	50.0 ml.	7	-	-	106.8	Observed for three-and-a-half months. Killed 2/5/56. No cysts seen in skin sections.	R 1497 R 1498	++ ++

G. = Goat; Sh. = Sheep; iv. = intravenously. + = mild; ++ = severe.

APPENDIX TABLE E.

DURATION OF BLOOD PARASITAEMIA IN ARTIFICIALLY *B. besnoiti* INFECTED RABBITS.

Rabbit No.	Donor No.	Date of infection.	No. of days after infection.	Dose of blood i.p. in ml.	Incubation period in days	Examination of blood smears		Clinical symptoms		Duration of disease in days	Severity of reaction	Result
						Date	Parasites	Oedema	Max. temp. °F.			
1318	1307	11.1.55	-	5.0	5	21.1.55	+	+	106.6	8	++	Recovered.
						22.1.55	+					
1321	1318	13.1.55	2	5.0	18	7.2.55	++	-	106.4	10	+++	Died.
						8.2.55	+++					
						9.2.55	+++					
1324	1318	15.1.55	4	5.0	12	29.1.55	+	+	106.0	5	++	Died.
						31.1.55	++					
1325	1318	17.1.55	6	5.0	12	1.2.55	+	+	105.8	4	++	Died.
1326	1318	19.1.55	8	5.0	8	31.2.55	++	+	107.0	6	++	Died.
						1.2.55	+++					
1330	1318	21.1.55	10	5.0	9	3.2.55	+	+	106.0	8	++	Died.
						4.2.55	+					
						5.2.55	+					
1331	1318	23.1.55	12	5.0	7	2.2.55	+	+	106.2	9	++	Died.
						3.2.55	++					
						4.2.55	+					
						5.2.55	++					
1332	1318	25.1.55	14	5.0	9	8.2.55	++	+++	107.4	8	+++	Died.
						9.2.55	+++					
						10.2.55	+++					
1333	1318	27.1.55	16	5.0	11	12.2.55	+	+	106.0	9	++	Died.
						14.2.55	+					
1334	1318	29.1.55	18	5.0	14	17.2.55	++	++	106.6	7	+++	Died.
						18.2.55	++					
1335	1318	31.1.55	20	5.0	21	28.2.55	++	+	105.6	8	++	Recovered.
						1.3.55	+					
1340	1318	4.2.55	22	5.0	18	23.2.55	+	-	105.2	7	++	Recovered.
1341	1318	4.2.55	24	5.0	23	2.3.55	+	+	105.8	10	++	Recovered.
						3.3.55	++					
						4.3.55	+++					

APPENDIX TABLE E (Continued).

DURATION OF BLOOD PARASITAEMIA IN ARTIFICIALLY *B. besnoiti* INFECTED RABBITS.

Rabbit No.	Donor No.	Date of infection.	No. of days after infection.	Dose of blood in ml.	Incubation period in days	Examination of blood smear		Clinical symptoms		Duration of disease in days.	Severity of reaction	Result
						Date	Parasites	Oedema	Max. Temp. °F			
:1436	:1431	:29.10.55	-	: 5.0	: 12	:15.11.55	++	-	: 106.0	: 10	++	:Redovered.
						:16.11.55	++					
:1457	:1436	:15.12.55	47	: 5.0	-	-	-	-	: 103.8	-	-	:No reaction.
	:1493	:15.2.56		: 5.0	8	:22.2.56	+	-	: 106.0	8	++	:Recovered.
:1458	:1436	:15.12.55	47	: 5.0	-	-	-	-	: 103.0	-	-	:Died during in- :cubation period.

\* Immunity test

Parasites + very rare  
++ rare  
+++ infrequent

Oedema + = mild  
++ = fairly severe  
+++ = very severe

Severity of reaction ++ = Fairly severe.  
+++ = Severe

TRANSMISSION OF *B. besnoiti* BY MEANS OF MATERIALS, OTHER THAN BLOOD, FROM AFFECTED RABBITS.

Rabbit No.	Donor No.	Date of Infection	Material used.	Dose in ml.	Route of administration	Incubation period in days	Examination of blood smears Date	Parasites	Clinical Symptoms. Oedema	Max. Temp. °F.	Duration of disease in days	Severity of Reaction	Result
13	RT	15.2.54	Oedematous fluid	5.0	sc.	6	25.2.54 26.2.54	+	+	106.2	5	++	Died.
1308	1296	4.1.55	Spleen suspension	5.0	ip.	7	15.1.55 17.1.55 18.1.55 20.1.55	+	+	106.6	7	++	Recovered.
1309	1296	4.1.55	Spleen suspension	5.0	ip.	7	15.1.55 16.1.55 17.1.55	+++	++	107.0	5	+++	Recovered.
1654	1641,1643	14.9.56	Urine and faeces	20.0	per os	-	-	-	-	103.0	-	-	No reaction.
1655	1641,1643	14.9.56	Urine and faeces	20.0	per os	-	-	-	-	103.6	-	-	No reaction.
1656	1641,1643	14.9.56	Urine and faeces	20.0	per os	-	-	-	-	103.2	-	-	No reaction.
1657	1641,1643	14.9.56	Urine and faeces	20.0	per os	-	-	-	-	103.2	-	-	No reaction.
1659	1641,1643	14.9.56	Liver and spleen suspension	5.0	ip.	10	30.9.56 1.10.56	++	+	105.6	8	++	Recovered.
1660	1641,1643 1681	14.9.56 *16.10.56	Liver and spleen suspension Blood	10.0 5.0	per os ip.	- 6	- 23.10.56	- +	-	102.8 106.0	- 8	- ++	No reaction. Recovered.
1661	1641,1643 1681	14.9.56 *16.10.56	Liver and spleen suspension Blood	10.0 5.0	per os ip.	- 7	- 24.10.56	- +	-	103.2 106.4	- 7	- ++	No reaction. Recovered.
1675	1668 1695,1696	8.10.56 *9.11.56	Faeces and urine Blood	30.0 5.0	per os ip.	- 8	- 21.11.56	- +++	-	104.0 106.0	- 8	- ++	No reaction. Recovered.
1676	1668 1696,1696	8.10.56 *9.11.56	Faeces and urine Blood	30.0 5.0	per os ip.	- 6	- 21.11.56 22.11.56	- +++ ++	-	103.4 106.2	- 9	- ++	No reaction. Recovered.

\*Immunity test.

Parasites + = very rare  
++ = rare  
+++ = infrequent

Oedema + = mild  
++ = fairly severe

Severity of reaction ++ = Fairly severe.  
+++ = Severe

APPENDIX TABLE G.  
SPERMATOGENESIS IN ARTIFICIALLY INFECTED RUMINANTS BEFORE AND AFTER RECOVERY

Animal	Donor	Date of infection	Dose of blood in ml.	Incubation period in days.	Examination of semen					Remarks.
					Date	Ejaculate in ml.	Motility	Percentage live sperms	Percentage abnormal sperms.	
Bull 5873	Rabbit 1522	24/4/56	35.0 iv.	6	24/4/56	8.0	Good	68	5	Developed shock after blood injection.
					27/4/56	8.0	Good	68	5	Highest temperature recorded 106.00F.
					1/5/56	5.0	0	0	0	
					8/5/56	6.0	0	0	All dead	
					15/5/56	5.5	0	0	All dead	
					22/5/56	4.0	0	0	All dead	
					28/5/56	4.0	0	0	No sperms	
					5/6/56	6.0	0	0	No sperms	<u>Trichomonas foetus</u> present.
					12/6/56	7.0	0	0	No sperms	‡ " "
					19/6/56	8.0	0	0	Few dead	" " "
					26/6/56	8.0	0	0	Few dead	" " "
					10/8/56	10.0	0	5	5	" " "
					14/9/56	12.0	0	5	5	" " "
					13/10/56	10.0	Poor	12	12	" " "
					30/10/56	7.0	Fair	50	0	" " "
6/12/56	8.0	Fair	55	0	" " "					
21/12/56	-	-	-	-	" " "					
Rabbit 1846	Rabbit 1846	15/5/57 <sup>‡</sup>	35.0 iv.	-	-	-	-	-	-	-
					22/5/57	5.0	Good	60	5	<u>Trichomonas foetus</u> absent
					29/5/57	6.0	Fair	63	5	" " "
					7/6/57	6.0	Fair	65	0	" " "
					13/6/57	7.0	Fair	61	0	" " "
					20/6/57	6.0	Fair	66	5	" " "
					4/7/57	7.0	Fair	63	0	" " "
8/8/57	7.0	Fair	75	0	" " "					
Rabbit 2350	Rabbit 2350	10/4/59 <sup>‡</sup>	30.0 sc.	-	10/4/59	-	-	-	-	No clinical symptoms seen.

‡ = Immunity Test.

APPENDIX TABLE G (Cont.)

SPERMATOGENESIS IN ARTIFICIALLY INFECTED RUMINANTS BEFORE AND AFTER RECOVERY.

Animal	Donor	Date of infection	Dose of blood in ml.	Incubation period in days.	Examination of semen					Remarks.
					Date	Ejaculate in ml.	Motility	Percentage live sperms	Percentage abnormal sperms	
Bull 5981	Rabbits 1512 and 1513	26/3/56	30.0 iv.	7	12/3/56	5.0	Good	65	0	Highest temperature recorded 106.2° F.
					15/3/56	5.0	Good	65	0	Animal listless during period of reaction.
					10/4/56	5.0	0	0	0	
					17/4/56	5.0	0	0	60	Left testicle swollen and hard.
					23/4/56	3.5	0	0	60	Swelling increased; animal dejected and shows stiff gait.
					1/5/56	4.0	0	0	No sperms	
					8/5/56	3.0	0	0	No sperms	
					15/5/56	4.5	0	0	No sperms	
					22/5/56	3.0	0	0	No sperms	
					12/6/56	6.0	0	0	Few dead.	
					19/6/56	8.0	0	0	Few dead	
					26/6/56	6.0	0	0	Few dead	Abscess opened; diagnosis <u>Corynebacterium</u> infection.
					10/8/56	6.0	0	27	50	
					14/9/56	12.0	Good	70	50	
13/10/56	7.0	Good	65	0	Left testicle fibrotic.					
30/10/56	10.0	Fair	63	0						
6/12/56	6.0	Fair	75	0						

APPENDIX TABLE G (Cont.)

SPERMATOGENESIS IN ARTIFICIALLY INFECTED RUMINANTS BEFORE AND AFTER RECOVERY.

Animal	Donor	Date of infection	Dose of blood in ml.	Incubation in days	Examination of Semen				Remark	
					Date	Ejaculate in ml.	Motility	Percentage live sperms		Percentage abnormal sperms
Billy Goat 3171	Rabbit 1922	21/9/57	30.0 iv.	6	21/9/57	1.0	Very good	76	0	Highest temperature recorded 106.8° F. Animal was listless, and showed inappetance for three days after height of thermal reaction.
					7/10/57	1.5	Poor	36	50	
					14/10/57	1.0	Poor	38	50	
					23/10/57	1.0	0	0	No sperms	
					28/10/57	1.0	0	0	No sperms	
					11/11/57	0.5	0	0	No sperms	
					19/11/57	2.0	Poor	30	59	
					2/12/57	1.0	Fair	49	49	
Rabbit 1977	23/1/57*	30.0 iv.	-	6/1/57	2.0	Good	70	0		
				7/2/58	2.5	Good	78	0		
				18/2/58	1.0	Good	75	0		
Rabbit 2307	19/3/59*	30.0 iv.	-	-	-	-	-	-	No clinical symptoms.	

APPENDIX TABLE G (Cont.)

SPERMATOGENESIS IN ARTIFICIALLY INFECTED RUMINANTS BEFORE AND AFTER RECOVERY.

Animal	Donor	Date of infection	Dose of blood in ml.	Incubation period in days	Examination of semen					Remarks.
					Date	Ejaculate in ml.	Motility	Percentage live sperms	Percentage abnormal sperms	
Ram 1952	Rabbit 1903	22/8/57	30.0 iv.	7	22/8/57	1.5	Very good	80	0	Highest temperature recorded 106.2° F. No further clinical symptoms.
					5/9/57	3.0	Very good	30	50	
					20/9/57	1.5	0	0	75	
					28/9/57	2.0	0	0	90	
					7/10/57	2.0	0	0	90	
					14/10/57	2.0	0	0	No sperms	
					23/10/57	2.0	0	0	0	
					28/10/57	0.5	0	0	0	
					11/11/57	0.75	0	30	75	
					19/11/57	1.0	Fair	30	35	
					2/12/57	2.0	Fair	60	35	
					6/1/57	2.0	Very good	80	0	
						Rabbit 1977	23/1/58*	30.0 iv.	-	7/2/57
	Rabbit 2338	2/4/59*	30.0 iv.	-	18/2/57	3.0	Good	70	0	
					-	-	-	-	-	No clinical reaction.

APPENDIX TABLE H.

RESPONSE OF ARTIFICIALLY B. BESNOITI INFECTED RABBITS TO TREATMENT DURING PERIOD OF REACTION.

Rabbit No.	Donor No.	Date of infection	Blood in ml.	Incubation period in days	Examination of blood smears		Clinical symptoms		Duration of disease in days	Treatment			Severity of disease	Remarks.
					Date	Parasites	Oedema	Max. temp. of		Drug	Dose per Kg. body weight	Method		
5	67	20/4/54	3.0 ip.	7	29/4/54 30/4/54	+	+	105.4	4	Phenergan Sulfamerazine	0.5 ml daily 500 mgm followed by 200 mgm daily for 2 days.	sc. per os	++	Died
6	67	20/4/54	3.0 ip.	10	1/5/54	+	+	105.0	5	Phenergan Sulfamerazine	0.5 m. daily 500 mgm followed by 200 mgm for 5 days	sc. per os	++	Died
54	39	19/3/54	2.5 ip.	8	29/3/54 31/3/54 1/4/54	+	+	106.2	4	Phenergan Sulfamerazine	0.75 ml. daily 600 mgm followed by 300 mgm daily for 2 days.	sc. per os	++	Died
55	39	19/3/54	2.5 ip.	12	31/3/54 1/4/54	+	+	105.4	8	Phenergan Sulfamerazine	0.75 ml. daily 600 mgm followed by 300 mgm daily for 2 days.	sc. per os	++	Died
57	49	26/3/54	2.5 ip.	8	7/4/54 8/4/54 9/4/54	+	+	105.8	7	Sulfamerazine	300 mgm daily for 4 days	per os	++	Recovered.
	96 and 98	19/5/54	5.0 ip.	-	-	-	-	-	-	-	-	-	-	Immune.
58	54	31/3/54	2.0 ip.	6	7/4/54	++	++	106.0	4	Sulfamerazine	400 mgm for 1 day	per os	++	Died.
59	54	31/3/54	2.0 ip.	6	9/4/54	+	+	106.6	5	Sulfamerazine	400 mgm daily for 2 days	per os	++	Died
60	54	31/3/54	2.0 ip.	7	10/4/54	+++	+++	106.8	3	Sulfamerazine	400 mgm for 1 day	per os	+++	Died
61	54	31/3/54	2.0 ip.	7	9/4/54 10/4/54	+	+	105.4	4	Sulfamerazine	400 mgm daily for 2 days	per os	++	Died
62	54	31/3/54	2.0 ip.	8	9/4/54	+	+	106.4	7	Sulfamerazine	400 mgm daily for 4 days	per os	++	Recovered
	96 and 98	19/5/54	5.0 ip.	-	-	-	-	102.8	-	-	-	-	-	Immune
64	56	7/4/54	2.5 ip.	6	14/4/54	+	+++	106.2	7	Sulfamerazine	400 mgm for 1 day	per os	++	Recovered.
65	56	7/4/54	2.5 ip.	6	14/4/54	+	+++	106.2	25	Sulfamerazine	400 mgm daily for 4 days	per os	++	Died

\* = Immunity test

Ø Rabbit unless otherwise stated.

APPENDIX TABLE H (Cont.)

RESPONSE OF ARTIFICIALLY B. BESNOITI INFECTED RABBITS TO TREATMENT DURING PERIOD OF REACTION.

Rabbit No.	Donor No.	Date of infection	Blood in ml.	Incubation period in days	Examination of blood smears		Clinical symptoms		Duration of disease in days	Treatment			Severity of disease	Remarks.
					Date	Parasites	Oedema	Max. temp. of		Drug	Dose per Kg. body weight	Method		
75	67	20/4/54	3.0 ip.	9	30/4/54 1/5/54	+	+	105.4	4	Sulfamerazine	400 mgm daily for 4 days	per os	++	Died
76	67	20/4/54	5.0 ip.	9	1/5/54	+	-	106.4	7	Sulfamerazine	400 mgm daily for 5 days	per os	++	Recovered.
77	67	20/4/54	3.0 ip.	8	-	-	-	104.8	7	Sulfamerazine	400 mgm daily for 5 days	per os	+	Recovered.
78	67	20/4/54	3.0 ip.	9	-	-	-	105.0	6	Sulfamerazine	400 mgm daily for 5 days	per os	+	Recovered
79	67	20/4/54	3.0 ip.	10	30/4/54 1/5/54	+	+	105.8	5	Sulfamerazine	400 mgm daily for 5 days	per os	++	Died.
84	80	29/4/54	2.5 ip.	8	10/5/54	+	+	104.0	4	Sulfamerazine	400 mgm for 1 day	per os	++	Died
85	80	29/4/54	2.5 ip.	11	13/5/54 14/5/54	+	-	106.4	6	Sulfamerazine	400 mgm daily for 3 days	per os	++	Died
39	24	4/3/54	2.5 ip.	13	19/3/54 20/3/54	+	+	107.0	3	Sulfamezathine (16% solution)	1.0 ml. daily for 2 days	iv.	++	Died
41	30	6/3/54	2.5 ip.	11	17/3/54 18/3/54	+	+	104.8	4	Sulfamezathine	1.0 ml. daily for 2 days	iv.	++	Died
42	30	6/3/54	10.0 sc.	8	16/3/54 17/3/54	+	+	106.2	7	Sulfamezathine	1.0 ml. daily for 3 days	iv.	++	Recovered
96 and 98	19/5/54	5.0 ip.	-	-	-	-	-	103.2	-	-	-	-	-	Immune
43	5870 ox	6/3/54	3.0 ip.	12	22/3/54	++	+	105.6	4	Sulfamezathine	1.5 ml. for 1 day	iv.	++	Died.
46	28	8/3/54	3.0 ip.	5	16/3/54 17/3/54 18/3/54	++	+++	106.6	6	Sulfamezathine	1.5 ml. daily for 2 days	iv.	+++	Died
47	28	8/3/54	6.0 sc.	9	20/3/54	+	-	106.0	7	Sulfamezathine	1.5 ml. for 1 day	iv.	++	Recovered
48	5870 ox	10/3/54	3.0 ip.	12	22/3/54 23/3/54 24/3/54	+	+	106.2	9	Sulfamezathine	1.5 ml. daily for five days	iv.	++	Died
49	5870 ox	10/3/54	3.0 ip.	13	26/3/54 27/3/54 29/3/54	+	+	106.0	7	Sulfamezathine	1.5 ml. daily for 5 days	iv.	++	Died
50	35	12/3/54	2.5 ip.	7	22/3/54 23/3/54	+	+	106.6	5	Sulfamezathine	1.5 ml. daily for 3 days	iv.	++	Died

APPENDIX TABLE H (Cont.)

RESPONSE OF ARTIFICIALLY *B. BESNOITI* INFECTED RABBITS TO TREATMENT DURING PERIOD OF REACTION.

Rabbit No.	Donor No.	Date of infection	Blood in ml.	Incubation period in days	Examination of blood smears		Clinical Symptoms		Duration of disease	Treatment			Severity of reaction	Remarks
					Date	Para-sites	Oedema	Max. temp. of		Drug	Dose per Kg body weight	Method		
52	40	12/3/54	2.5 ip.	7	23/3/54 24/3/54	+ +	+ +	106.4	6	Sulfamezathine	1.5 ml daily for 2 days	iv.	++	Died
86	80	29/4/54	2.5 ip.	6	-	-	-	105.6	15	Sulfamezathine	1.5 ml. daily for 3 days	iv.	+	Died
87	80	29/4/54	2.5 ip.	6	-	-	-	106.6	6	Sulfamezathine	1.5 ml. for 1 day	iv.	+	Died
19	1	16/2/54	2.5 ip.	6	24/2/54 25/2/54 26/2/54 27/2/54	++ ++ ++ ++	++ ++ ++ ++	105.2	13	Aureomycin (1% solution)	10.0 mgm. daily for 5 days	iv.	+++	Died
25	2	18/2/54	6.0 ip.	5	26/2/54 27/2/54 28/2/54 1/3/54	+++ +++ +++ +++	++ ++ ++ ++	106.2	7	Aureomycin	10.0 mgm. daily for 3 days	iv.	++	Died
31	18	25/2/54	3.0 sc.	11	9/3/54	++	+	105.0	3	Aureomycin	10.0 mgm. daily for 3 days	iv.	++	Died
33	19	27/2/54	3.0 ip.	9	8/3/54	++	++	105.6	3	Aureomycin	10.0 mgm. daily for 2 days	iv.	+++	Died
92	89	8/5/54	2.5 ip.	6	18/5/54	++	+	106.2	10	Phenergan Achromycin (1% solution)	0.5 ml daily 10.0 mgm daily for 4 days.	im. iv.	++	Died
93	83	10/5/54	2.5 ip.	6	19/5/54	++	+++	105.4	6	Phenergan Achromycin	0.5 ml. daily 10.0 mgm. daily for 3 days	im. iv.	++	Died
95	83	10/5/54	2.5 ip.	7	22/5/54	++	-	105.0	11	Phenergan Achromycin	0.5 ml. daily 10.0 mgm daily for 4 days	im. iv.	++	Died
96	83	10/5/54	2.5 ip.	7	19/5/54	+	-	105.6	52	Phenergan Achromycin	0.5 ml. daily 10.0 mgm. daily for 4 days	im. iv.	++	Died
97	83	10/5/54	2.5 ip.	6	-	-	+	104.8	4	Phenergan Achromycin	0.5 ml 10.0 mgm. for one day	im. iv.	+	Died
98	83	10/5/54	2.5 ip.	9	19/5/54 20/5/54	++ ++	- -	105.2	7	Phenergan Achromycin	0.5 ml daily 10.0 mgm. daily for 4 days.	im. iv.	++	Recovered

APPENDIX TABLE H.

RESPONSE OF ARTIFICIALLY B. BESNOITI INFECTED RABBITS TO TREATMENT DURING PERIOD OF REACTION.

Rabbit No.	Donor No.	Date of infection	Blood in ml.	Incubation period in days	Examination of blood smears		Clinical symptoms		Duration of disease in days	Treatment			Severity of disease	Remarks
					Date	Parasites	Oedema	Max. temp. of		Drug	Dose per Kg. body weight	Method		
34	19	27/2/54	3.0 sc.	12	12/3/54 13/3/54 14/3/54	+ + +	+++	105.2	5	Babesin (Hoechst)	3.0 mgm for 1 day	iv.	+++	Died
35	19	27/2/54	3.0 sc.	10	12/3/54	++	+	105.0	5	Babesin	4.0 mgm for 1 day	iv.	++	Died
38	24	4/3/54	2.5 ip.	7	12/3/54 13/3/54 15/3/54	+ + +	+	106.0	7	Gonacrine (5% solution)	0.05 mgm for 1 day	iv.	+++	Died
40	30	6/3/54	2.5 ip.	6	12/3/54 13/3/54 14/3/54	+ + +	+	105.8	5	Gonacrine	0.05 mgm for 1 day	iv.	+++	Died
1550	1539 & 1541	16/5/56	5.0 ip.	15	6/6/56 7/6/56	+ +	-	105.4	7	Pentamidine (1% solution)	1.5 ml. daily for 2 days	sc.	++	Recovered.
1552	1539 & 1541	16/5/56	5.0 ip.	14	5/6/56	+	+	105.4	15	Pentamidine	2.0 ml. for 1 day	sc.	++	Died
1553	1539 & 1541	16/5/56	5.0 ip.	14	7/6/56	++		105.0	7	Pentamidine	2.5 ml. daily for 2 days	sc.	++	Recovered
80	67	20/4/54	3.0 ip.	6	29/4/54 30/4/54	+ +	+	105.6	5	Phenamidine (5% solution)	0.4 ml. for 1 day	sc.	++	Died.
81	65	21/4/54	3.0 ip.	7	1/5/54	+	-	105.8	8	Phenamidine	0.4 ml. daily for 2 days	sc.	++	Died.
82	65	21/4/54	3.0 ip.	7	30/4/54 1/5/54	+ +	+	106.0	3	Phenamidine	0.4 ml. for 1 day	sc.	++	Died.
89	74	29/4/54	2.5 ip.	5	8/5/54	+	-	106.8	7	Phenamidine	0.5 ml. daily for 2 days	sc.	++	Recovered.
90	74	29/4/54	2.5 ip.	5	-	-	-	106.4	10	Phenamidine	0.5 ml. daily for 2 days	sc.	+	Recovered.
88	80	29/4/54	2.5 ip.	5	10/5/54	+	-	106.0	7	Plasmoquine (16% solution)	0.5 ml. for 1 day	iv.	++	Died.
1251	79328 (goat)	12/10/54	6.0 ip.	13	27/10/54 28/10/54 29/10/54	+ + +	++	107.0	12	Sodium iodide (10% solution)	2.5 ml. twice at 3 daily intervals	iv.	+++	Died
1252	79328 (goat)	12/10/54	6.0 ip.	9	25/10/54 27/10/54 28/10/54	+ + +	+	106.0	13	Sodium iodide	2.5 ml for 1 day	iv.	++	Died

APPENDIX TABLE I.

REPEATED TREATMENT OF 26 RABBITS DURING INCUBATION PERIOD, CONTINUED IN 19 CASES ALSO DURING REACTION PERIOD.

Rabbit No.	Donor	Date of infection 5.0 ml. blood ip.	Controls Table C	Incubation period in days	Examination of blood smears		Clinical symptoms		Duration of disease in days	Treatment			Severity of reaction	Result.	
					Date	Parasites	Oedema	Max. temp. of		Drug.	Dose per Kg body weight	Days after infection			Method
1566	1556	30/5/56	Gen.76	16	19/6/57	+	+	106.0	8	Trypan blue (1% solution)	4.0 mgm	2,4,6,8,10,12,14,16	iv.	++	Recovered
1567	1556	30/5/56	Gen.76	8	10/6/57	+	-	105.4	7	Pentamidine (1% solution)	0.5 ml.	2,4,6,8,10,12	sc.	++	Recovered
1568	1556	30/5/56	Gen.76	10	13/6/57	-	++	106.0	8	Pentamidine	1.0 ml	2,4,6,8,10,12,14,16,18,20,22	sc.	++	Recovered
1569	1556	30/5/56	Gen.76	10	13/6/57	++	+	105.0	9	Mycostatin Phenergan	500,000 units 0.75 ml.	2,4,6,8,10,12	per os im.	++	Died
1583	1563	15/6/56	Gen.77	10	-	-	+	107.0	5	Phenamidine (5% solution)	0.6 ml	2,4,6,8,11,13,15	sc.	++	Died
1584	1563	15/6/56	Gen.77	7	2/7/56	++	-	105.4	11	Anthiomaline	0.04 ml	3,6,10,13	im.	++	Died
1630	1614	20/8/56	Gen.82	7	31/8/56	+	-	105.0	7	Pamaquin	0.2 ml.	2,4,6,8	im.	++	Recovered
1631	1614	20/8/56	Gen.82	9	4/9/56	+	-	106.2	8	Aralen	0.4 ml	2,4,6,8	im.	++	Recovered
1632	1614	20/8/56	Gen.82	77	-	-	+	106.6	5	Aralen	100 mgm	2,4,6,8	per os	++	Died
1684	1668	8/10/56	Gen.86	9	20/10/56	++	+	104.4	5	Mixtamycin	400,000 units	2,4,6,8	im.	++	Died
1686	1668	8/10/56	Gen.86	8	20/10/56	++	++	104.8	6	Mixtamycin Phenergan	400,000 units 0.75 ml	2,4,6,8	im. im.	++	Died
1685	1668	8/10/56	Gen.86	15	-	-	-	105.0	7	Terramycin	10.0 mgm	2,4,6,8	iv.	+	Recovered
	1696	9/11/56		-	-	-	-	103.0	-	-	-	-	-	-	Immune
1707	1695	9/11/56	Gen.89	14	28/11/56	++	+	103.8	5	Terramycin	20.0 mgm	3,4,5,6,7,8	iv.	++	Died
1724	1714	1/12/56	Gen.91	9	13/12/56	+	-	105.0	8	Quinacrine	9.0 mgm	3,4,5,6,7,8	per os	++	Recovered
1725	1714	1/12/56	Gen.91	9	14/12/56	+	-	105.6	7	Quinacrine	9.0 mgm	3,4,5,6,7,8	per os	++	Recovered
1726	1714	1/12/56	Gen.91	8	13/12/56	++	-	105.8	7	Quinacrine	9.0 mgm	3,4,5,6,7,8	per os	++	Recovered
1912	1907	22/8/57	Gen.115	7	4/9/57	++	-	107.0	9	Spirotrypan	0.2 ml.	2,4,6,8,10,12	im.	+++	Died
1913	1907	22/8/57	Gen.115	8	10/9/57	+	-	105.0	7	Spirotrypan	0.4 ml.	2,4,5,8,10,12	im.	+	Recovered
1914	1907	22/8/57	Gen.115	7	8/9/57	+	-	105.0	7	Spirotrypan	0.4 ml	2,4,6,8,10,12	im.	++	Recovered
1915	1907	22/8/57	Gen.115	8	-	-	+	105.6	10	Spirotrypan	0.6 ml	2,4,6,8,10,12	im.	++	Died
1928	1920	21/9/57	Gen.117	7	2/10/57	+	-	106.0	7	Formalin (1% solution)	0.4 ml.	2,4,6,8	iv.	++	Recovered

⊛ Immunity Test

APPENDIX TABLE I (Cont.)

REPEATED TREATMENT OF 26 RABBITS DURING INCUBATION PERIOD, CONTINUED IN 19 CASES ALSO DURING REACTION PERIOD.

Rabbit No.	Donor	Date of infection 5.0 ml. blood ip.	Controls Table C	Incubation period in days	Examination of blood smears		Clinical symptoms		Duration of disease in days	Treatment			Severity of reaction	Remarks	
					Date	Parasites	Oedema	Max. temp. of		Drug	Dose per Kg body weight	Days after infection			Method
1929	1920	21/9/57	Gen.117	8	4/10/57	+	-	105.4	7	Formalin (1% solution)	0.4 ml.	2,4,6,8	iv.	++	Recovered.
1930	1920	21/9/57	Gen.117	6	1/10/57	+	+	104.8	7	Formalin	0.6 ml.	2,4,6,8	iv.	++	Recovered.
1931	1920	21/9/57	Gen.117	5	2/10/57	+	-	105.0	7	Formalin	0.8 ml.	2,4,6,8	iv.	++	Recovered.
1950	1944	2/11/57	Gen.121	8	13/11/57	+	-	105.0	8	Tubomel(INH) P.A.S.	20.0 mgm 200 mgm	3,4,5,6; 7,8.	per os	++	Recovered.
1951	1944	2/11/57	Gen.121	9	13/11/57 14/11/57	++ +	+	105.4	8	Tubomel(INH) P.A.S.	20.0 mgm. 200 mgm.	3,4,5,6; 7,8.	per os	++	Recovered.

Parasites: + = rare

++ = infrequent

+++ = frequent

Oedema: + = mild

++ = severe

+++ = very severe

Severity: + = mild

++ = severe

+++ = very severe