THE DEVELOPMENT OF THEILERIA PARVA, THE CAUSE OF EAST COAST FEVER OF CATTLE IN SOUTH AFRICA.

By Dr. Richard Gonder, of the Institut für Tropen and Schiffs Hygiene, Hamburg (at the Veterinary Bacteriological Laboratory, Onderstepoort, Pretoria, South Africa).

In a preliminary report* at the end of April I briefly mentioned the development of the parasite of East Coast fever in the organs of cattle in South Africa, and I particularly emphasized that *Piroplasma mutans* and *Theileria parva* (*Piroplasma parvum, Babesia parva, vide* next paragraph), although they are from a morphological point of view difficult to distinguish, are not identical, the latter being the cause of East Coast fever; and I showed that this parasite, before it appears in the blood of the sick animal, has to undergo certain stages of development in the organs and especially in the lymphatic glands and in the spleen. The various stages of development of *Theileria parva* are easily demonstrated in material obtained by means of a puncture of the spleen or lymphatic glands and represent the so-called Koch's granules or plasma bodies. Certain phases of the latter bodies present typical forms from which, by schizogony, the well-known East Coast fever parasites develop and then become visible in the blood corpuscles.

Having been able to confirm these observations by further experiments, I will now, as far as possible, show the developmental stages of the cycle of the East Coast fever parasites by means of drawings and microphotographs, but I wish to point out that I have not been able to trace all the forms of that cycle. Finally I will touch briefly upon their further development in the ticks.

Concerning the name *Babesia parva* (*Piroplasma parvum*), the cause of East Coast fever, I pointed out in the article referred to previously that *Bettencourt, Franca*, and *Borges* proposed a new name, raising it to the rank of a genus. As will be seen later, this parasite, both biologically and morphologically, is so different from the "Babesiae" that we must separate it from this genus. *Theiler* had already pointed out the biological difference when he separated *Babesia bigeminum* and *mutans* (*Pir. big.* and *mutans*) from *Theileria parva* (*Pir. parv.*). Biological qualities do not come into final consideration if we separate different genera or species of parasites, otherwise we would have to separate from each other a great many species of trypanosomes which morphologically belong together.

Bettencourt, Franca, and *Borges*, for morphological reasons, introduced the new name, viz., *Theileria parva*, a name which, since then, has been accepted by *Nuttall, Smith*, and *Fantham*. We must

* Berl. Tieraerztl. Wochenschrift, July, 1910, and Supplement to the Annals of the Transvaal Museum; Proceedings of the Biological Society, Pretoria, 1910; Royal Society of South Africa, July, 1910.

add, however, that *Bettencourt* and his collaborators had at that time not sufficient grounds to justify them in introducing a new name for this species, for the size which fluctuates in plasmodides and piroplasmides considerably, according to the stage of development they are in, and the formation of cross forms, cannot be considered to be sufficient for this purpose.

Extremely small parasites are found with *Babesia mutans* and with *Achromaticus vesperuginis*, both of which also form cross shapes and with certain blood parasites of monkeys, all of which have to be placed in the classification next to the East Coast fever parasites. The characteristic of our parasite is its course of development which will also determine the systematic position and which was at that time unknown to *Bettencourt* and his collaborators. I will also adopt the name of *Theileria parva*. The development forms of this parasite in the organs of cattle are so characteristic that they can be depended on for the diagnosis of the disease. They are specific for the parasite, and their presence could not be proved in other diseases caused by babesiae. The objections which *Martin Meyer* (Hamburg) raised regarding these forms of development, viz., Koch's bodies, considering them not specific for East Coast fever, require further investigations, and the views expressed by him have not been confirmed in any way by other investigators.

By observing a multiple segmentation of Koch's granules into a number of typical East Coast fever parasites, *Martin Meyer's* explanation, that we have to deal with reaction products, becomes invalid. In some cases of babesia infection now and again such reaction products may occur which, when treated with dry fixing and giemsa staining, may resemble certain forms of the stages of development of *Theileria parva*.

Robert Koch was the first who declared the East Coast fever of cattle in South Africa to be a disease *sui generis*, and who described and illustrated the parasite *Theileria parva*, and who mentioned the presence of peculiar bodies, probably stages of development, in the organs. *Lounsbury* and *Theiler* determined, by a series of experiments, the different kinds of ticks which come into consideration for the propagation of East Coast fever in South Africa, and they also give exact information regarding the incubation time of the disease and the biology of these ticks. *Theiler* then demonstrated the existence of another parasite similar to that of East Coast fever, which he called *Babesia mutans*, and which had been considered by *Koch* to be identical with *Theileria parva*. *Theiler* proved by experiments, which are still being continued, that these two parasites are from a biological point of view totally different—the one, *Babesia mutans*, is transmitted by blood and is often met with in the blood of South African cattle; the other. *Theileria parva*, cannot yet be conveyed by the injection of blood. *Theiler* recently succeeded in separating still another parasite from *Babesia bigeminum, mutans*, and *Theileria*, viz., *Anaplasma marginale*, the parasitic nature of which still requires further investigations. According to *Theiler, Anaplasma marginale*, hitherto known under the name of *marginal points*, causes a severe anaemia, often ending in death. *Theiler* holds anaplasma to be an organism reduced by parasitism, having lost its "organelles" and its plasma, accordingly it would phylogenetically represent one of the oldest piroplasmidae.

The South African cattle being exposed to so many protozoon diseases, it is evident that it took a considerable time before full light could be thrown on the morphological varieties of the different parasites.

On account of the great similarity between *Babesia mutans* and *Theileria parva* (in the blood), and considering that recovery from East Coast fever is succeeded by complete immunity, *Fuelleborn* and *Ollwig* doubted their non-identity. They thought, in common with others who have made a study of tropical disease, that East Coast fever is not a piroplasmosis, and, in opposition to most authors, that an invisible parasite conveyed by ticks might cause the disease, as is the case in yellow fever, pappataci fever, and other diseases carried by insects. Previously, *Robertson*, in the Cape Colony, held the view as to an invisible parasite being the cause of East Coast fever, because he had observed cases of East Coast fever where no visible parasites could be traced. As the earlier experiments had always been made with South African cattle, latent infections caused by *Babesia mutans* could not be excluded, and thus the latter authors had some reasons to maintain their views.

In my preliminary publication, I referred to some experiments which proved in an unmistakable way that *Theileria parva* and *Babesia mutans* are *not* identical.

These experiments had been made on English cattle freshly imported. Already *Stockmann* had previously made similar experiments in England with the same results. *Babesia mutans* is easily transmitted by the injection of small quantities of blood from one animal to another, which is impossible with *Theileria parva* in its endoglobular stage.

Kleine, *Lichtenheld*, *Theiler*, and *Walker* considered the stages of development in the organs, viz., Koch's granules, to be of practical use in the diagnosis of East Coast fever. *Lichtenheld* described different forms of these stages, *Kleine* and *Walker* tried to prove the specificity of these granules, all being of opinion that the granules represented distinct stages of development, although they have not been able to explain them.

In addition to this I ought to mention the Transcaucasian or tropical piroplasmosis and the so-called piroplasmosis (*Bitter*) of cattle in Egypt. *Dschunkowsky* and *Luhs* described a severe disease of cattle in Transcaucasia. They distinguish two forms, an acute and a chronic or cachectical one. Based on the reports of *Theiler*, contributed to the International Veterinary Congress of the Hague and on pictures and descriptions by *Dschunkowsky* and *Luhs*, I am of the same opinion as *Theiler* that we have to deal with double infections. *Dschunkowsky* and *Luhs* have drawn bodies which *Theiler* described to be *Anaplasma marginale*. Haemoglobinuria and *Babesia bigeminum* have also been found. Most probably *Babesia mutans* occurs in the blood of Transcaucasian cattle, in which country apparently similar conditions are met with as in South Africa. More investigation into the presence of ticks which are able to transmit East Coast fever is required. *Dschunkowsky* and *Luhs* mention *Rhiphicephalus sanguineus* and *bursa*, and since in South Africa all Rhiphicephalidae have been known to transmit East Coast fever this disease cannot be excluded in the Transcaucasus. Having examined some blood smears, for which I am indebted to Dr. Theiler, I must

admit that the parasites causing tropical piroplasmosis (*Pir. annulatum*), described by *Dschunkowsky* and *Luhs*, differ considerably from *Theileria parva*, the former having an annular shape. With *Theileria parva* ring forms do occur frequently, but not in great numbers, and mostly in the beginning of the disease. Also the pathological-anatomical condition, the forming of haemorrhages on tongue and eyes, indicate a disease which is slightly different from East Coast fever; after all it is perhaps only a variety of the latter. According to several reports from other sources Koch's granules occur in connection with this disease.

Double infections are often met with in Africa, in fact it is the usual thing. If *Dschunkowsky* and *Luhs* should succeed (as was the case with *Theiler*) in separating these parasites from each other by means of ticks and artificial transmission and obtain pure infections of each separate parasite, then it will be easier to discuss the Transcaucasian piroplasmosis and its cause, *Pir. annulatum*. The same may be said about Bitter's piroplasmosis in Egypt and about Ducloux's piroplasmosis bacilliforme in Tunis.

Material and Methods of Investigation.

Cattle used for experiments and parasitological investigations came partly from England, i.e. cattle not infected with *Babesia mutans*, *Theileria parva*, and *Anaplasma marginale*, and partly from non-East Coast fever or redwater infected areas of South Africa. Further, young calves born at this laboratory were kept isolated and under special conditions in order to exclude any tick infection. Also, young animals, which *Theiler* used as controls in his experiments on immunity were made use of. Thus it was possible to utilize a considerable number of cattle infected with *Theileria parva* for my studies. Regarding ticks, I had, in fact, the entire material of this institute used for East Coast fever investigations placed at my disposal. I had also an opportunity to examine the blood smears and organs from diseased and dead animals sent to us from other districts.

In order to be always sure that the ticks used were infected, principally those of the Rhiphicephalidae species were utilized, viz., *Rhiphicephalus appendiculatus*, as this tick is known to transmit East Coast fever almost without fail. *Rhiphicephalus evertsi* which was used in the beginning was soon discarded for the study of the further development in ticks. It is a well-established fact that the East Coast fever parasites are not propagated by larval ticks, as is the case with *Babesia bigemina*. Consequently ticks raised from larvae, if the former were not infected with *Babesia bigemina*, *Babesia mutans*, or *Spirochaete theileri*, must be clean. I was able to obtain clean ticks, and I infected *Rhiph. appendiculatus* in its larval and nymphal stages.

The first experiment was made with a healthy English heifer, to which East Coast fever was transmitted with mature ticks of *Rhiph. appendiculatus*, infected with *Theileria parva*. The result was that this heifer contracted typical East Coast fever and succumbed. Transmission of blood into a second English heifer, also not immune against redwater, did not cause this heifer to contract an infection with *Babesia bigemina*, *Babesia mutans*, or other parasites transmitted by blood. The first English heifer was infested with some thousands

of larvae and nymphae which were all collected in due time, and thus enough material was obtained for experiments.

In order to study the cycle of development microscopically, recourse was taken to the living object. Since the various forms of development in the organs do not offer any interesting phases on account of their immobility, with the exception of those which are in a stage of segmentation, I paid more attention to the schizogony of Koch's granules, which I often observed. The parasites found in the blood have, with the development to *Theileria parva*, reached a certain stage; they are so small and slow in their movements that we do not gain much information as to their morphology and biology for their study during life. It is different with ticks in the body of which a copulation of the East Coast fever parasites takes place, resulting in the formation of active ookinetes.

In order to get a preliminary idea concerning the degree of infection, the number and stages of development present in the glands, dry fixing and staining according to *Giemsa* was applied, and then, according to what was found further, cytological studies were carried out on the living object and after moist fixing and staining with various stains. When a sick animal was killed, or when it had succumbed to the disease, pieces of different organs were fixed and mounted in paraffin, and the parasites in the sections examined.

Giemsa stain after moist fixing with a concentrated sublimate solution rendered good service. In preparations thus fixed both generations showed off well both on glass smears and in the sections. I called the two stages of the cycle "agamogonous" and "gamogonous", according to *Hartmann*. Dry fixing does not show clearly the difference in the nuclei of these two stages.

For the study of these nuclear phenomena, I can recommend *Schaudinn's* solution, sublimate and acetic glacial alcohol and staining with a mixture of haematoxyline by *Heidenhain*, *Delafield*, and *Ehrlich*. I further made use of *Flemming's* and *Hermann's* mixtures for fixing purposes, and for the fixation of the haemolymphatic glands and lung pieces I used a mixture for which I am indebted to the late *Dr. Lo Bianco*: *Flemming* (strong) and formol (conc.) 1 : 1. In the latter mixture the pieces are to be fixed only for a short time, then to be washed in water and to be kept in alcohol. Less successful was staining with *Borrel's* and *Mallory's* mixtures. Suitable solutions for the vital staining are neutral red (1 per cent.), methyl green, and methylene blue.

The parasites are so distinctly visible in the glands and other organs that vital staining will only be necessary if reagents like quinine, etc., are used. The juice of glands and spleen are easily obtainable by means of a puncture made with the canula of an ordinary syringe. Small pieces of organs which adhere to the canula are very suitable for examinations.

Life Cycle of Theileria parva.

Up to now I have succeeded in observing only the formation of living ookintes in the tick *Rhiphicephalus appendiculatus*. However, I hope to be able to trace the copulation at a later date. A further development within the tick is certain. For besides characteristic forms in the stomach of the tick (I cannot say yet whether they are microgametes or macrogametes) I have discovered extremely small

parasites collected in clusters. I found these forms neither in purified ticks nor in non-infected or control ticks. I believe that we have to deal with *sporozoites* in this case. These forms are only to be found in the nymphal stage of the tick after the larva has been infected, or in the adult tick after the nymph has been infected, i.e. at a time when the tick is looking for another host. If the infected tick gets hold of a healthy animal, the latter becomes infected. I never succeeded in tracing the existence of these forms in the body of cattle. Likewise I could not convey the infection by an intracutaneous, intralymphal, or by any other way with emulsions or salivary glands or gastric juice of infected ticks. I cannot give yet an explanation for this. Perhaps the external temperature influences this in some way or the further development of these sporozoites within the tick is only possible after it has been sucking blood. The tick which remains a shorter or longer period on a beast until it is repleted, cleans itself completely from infection. For this latter purpose not only cattle may be of use but also sheep or rabbits. Ticks infected with *Theileria parva*, which transmitted this infection to cattle, and ticks of the same lot, fed on rabbits, could no longer transmit East Coast fever when again placed on a susceptible animal.

The first stages which are found in cattle are mostly small extra globular round forms with one single nucleus. If the preparations are examined carefully, these forms will generally be observed on about the tenth day after infestation of ticks in the lymphatic glands or in the spleen. A few days later they are also found to be intracellular in the large mononuclear lymphocytes. Soon the parasite increases in size, the number of nuclei increases considerably, and finally the parasite breaks into as many parts as there are nuclei. This is the usual way, in the case of the intracellular parasites, the host cell the lymphocyte, disintegrates earlier than the parasite. This may result in irregular segmentation, and the particles may contain the merozoites or better called agamonts with two, three, or more irregularly shaped nuclei. This method of development is subject to repetition.

Out of this agamogonous generation—I would not like to say asexual, as from the beginning sexual differences might exist—a second generation comes forth, which I shall designate a gamogonous one. By multiplication and subsequent segmentation certain forms are liberated which are distinguished from the above-mentioned agamonts by their more regularly shaped and more compact nuclei. These forms, gamonts, are usually found shortly before the parasites of East Coast fever appear in the blood, generally eighteen days after the infestation with ticks. The nuclei of these forms take the stain more intensely than those of the agamogonous one. These parasites are found free as well as intracellularly. The young gamont, consisting of one nucleus only, increases rapidly from one day to another, but often divides, especially if it is placed extraglobularly, without having grown very much.

The intracellular gamonts furnish a far greater number of parasites, if the attacked lymphocyte does not die off too soon. In a later paragraph I will try to explain this fact. The parasites produced by the gamonts represent the East Coast fever parasites which enter into the blood corpuscles. With the formation of these forms, the gametocytes, the development of the parasite in cattle reaches a

definite point. It often happens that undeveloped gametocytes, which are still gamonts, enter the blood corpuscles and then continue their transformation. This is the reason why other authors refer to a binary division, and to a reproduction in the blood of cattle. Sometimes cross forms are likewise observed in the blood which appear in the usual way in the organs when segmenting, i.e. when undeveloped gametocytes, viz., gamonts, enter the blood corpuscles. However, ordinarily a reproduction in the blood does not take place.

It is a fact that no parasites are to be discovered in so-called salted cattle, i.e. immune against East Coast fever, and that the latter have, up till now, always been found not to be subject to relapse, which proves that the parasites must perish after a certain period, and that they cannot undergo parthenogenesis. It is also impossible to transmit East Coast fever by means of blood, a circumstance which speaks against a further development of parasites in the blood. Cattle recovering from the disease show agamogonous and gamogonous forms if the disease is at its height. The agamogonous form disappear very soon. If the number of parasites decreases in the blood, the number of gamogonous forms will lessen in the organs, i.e. if the gamogonous forms disappear in the organs, lymphatic glands, spleen, etc., the development of parasites in the body of the animal is arrested.

If these gametocytes manage to get into the stomach of a tick, the formation of gametes begins. A copulation takes place between microgametes and macrogametes (not yet observed); mobile ookintes are formed, out of which finally the above-mentioned sporozoites must come forth.

Agamogonous Generation.

In order to obtain a good idea of the development of the successive stages of our parasites, we must start with the beginning of the disease.

East Coast fever is first recognized by the fever, which increases day by day. At the height of the disease, and shortly before death, the organs, especially the lymphatic glands, are often crowded with all sorts of stages of development, so that it is difficult to distinguish this life cycle clearly. The disintegration of infected lamphocytes makes it more difficult still. I managed to get good results only by making punctures into different lymphatic glands (*Glandula cervicalis superficialis, Glandula precourales*), and, as far as possible, also into the spleen, and this was done very systematically, day by day.

At first we perceive mostly extra globular forms, with one nucleus and very little protoplasm. The nucleus of these youngest agamonts is irregularly shaped, and does not possess any nuclear envelope, nor is it clearly defined from the protoplasm. There are sometimes one or more small particles in the nucleus which absorb more intensely the staining material, and which I hold to be karyosomes. Generally, the nuclei show a peculiar honeycomb chromatine which does not possess any great affinity for nucleus-staining material. When the agamont begins to disintegrate, more or less distinctly stained chromatin nuclei appear. These nuclei are always in evidence where the formation of the next gamogonous generation commences.

The agamont increases in volume and the number of its nuclei also increases. The propagation of the nuclei which keeps pace with the growth of the parasites is generally effected amitotically by means of successive division (compare Plate I, Figs. 1-9 and 12-17). I could

discern (which is an exceptional case) on sections and in moist fixation an intimation of primitive mitosis, similar to cocidia and haemogregarine (Karyolysus).

There is an accumulation at the poles of chromatin particles which take staining more intensely. The division of the nucleus is caused by the karyosome which splits into portions. However, as mentioned before, I noticed this kind of division very rarely. Frequently the nucleus, or rather the different portions of nuclei, seemed to split into two or more particles. A similar process of division has been noticed in connection with other protozoa, the opalinides, the latter being of a parasitic nature. Similar conditions of agamogonous forms in the lungs and in the liver are met with in *Haemoproteus columbae*. By successive division the globular parasite obtains a very considerable number of nuclei, sometimes amounting to 70-80 (very rarely). The nuclei accumulate on the surface of the parasite, showing forms not unlike blackberries or stramonium, those of the latter appearing shortly before segmentation takes place (*Lichtenheld*). Finally, the parasite is divided into a number of irregularly shaped daughter formations (Plate I, Figs. 1 and 9; Plate IV, Figs. 6 and 9).

Agametes produced by this process (schizogony) frequently show a second nucleus. The formation of this second nucleus, a kind of blepharoplast, is of great importance regarding the classification of *Theileria parva*. Thus we have similar conditions, as observed with Haemoproteus and the malaria parasites of man, in which case a secondary nucleus very rarely occurs. The appearance of a second nucleus indicates a relationship with flagellates. Large extracellular forms, as illustrated on Plate I, Figs. 9-11, are not very frequently observed; schizogony generally takes place at an earlier stage of development (Plate I, Fig. 8; Plate IV, Figs. 5 and 6).

In the same way the development of the intracellular agamonts takes place, with the exception that a greater number of agametes are to be obtained (Plate I, Figs. 12-19; Plate VIII, Figs. 7-9). The reason for this may be found in the fact that the lymphocyte shows double infection (Plate I, Fig. 16), by which process naturally the number of agametes increases.

If the lymphocyte does not perish prematurely through the noxious influence of its parasite, i.e. before schizogony takes place, its plasma will be occupied entirely by the parasite. In most cases the segmentation of parasites means also the destruction of the lymphocyte (Plate I, Figs. 17-19). The age of the lymphocyte will be an important factor in resisting the parasite. Frequently we find the disintegration of the nuclei of lymphocytes or leucocytes, when infected with very young agamonts. Perhaps the above-mentioned large, free forms (Figs. 9 and 10) may possibly have been liberated by the destruction of the host cell and shortly before the parasite started to divide.

The plasma of all forms of the agamogonous generation is distinctly alveolar and honeycombed. In preparations stained according to *Giemsa*, two kinds of plasma may be distinguished at different stages, viz., the one being of a very pale blue colour and the other of a dark blue colour. This difference in the colour by the *Giemsa* staining is far more marked in intracellular agamonts, which in their youngest stage of development might easily be taken for cell granulations—for instance, azurophile granules—consequently one has to be careful not to make any mistakes. The protoplasma of the parasites is always