

Eperythrozoon Ovis (sp. nov.) Infection in Sheep.

By

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THE demonstration by Biffi (1903), Gastiaború (1903) and Barton (1905) of bacillary shaped bodies in the erythrocytes of patients suffering from Oroya fever and Verruga peruviana stimulated a great deal of work which has added immensely to the knowledge of the *Bartonella* group of organisms. Barton continued his observations and in 1909 reported that the intracellular organisms which he constantly found in patients were of the nature of protozoa and that he believed them to be the cause of Oroya fever or Carrion's disease. Subsequently these observations were confirmed by the American Commission consisting of Strong, Tyzzer, Brues, Sillard and Gastiaború (1913). However, many other workers did not regard these bodies as parasites, and brought forward to disprove their parasitic nature arguments similar to those advanced to discredit the parasitic conception of the *Anaplasmata*. Not until Noguchi (1926) showed that the *Bartonella* could be cultivated was their parasitic nature accepted. Finally Noguchi and Battestini (1927) and Mayer and Kikuth (1927) showed that there was a common aetiological agent responsible for Oroya fever and Verruga peruviana, which frequently follows Oroya fever, viz., *Bartonella bacilliformis*.

Parasites similar to the *Bartonella* found in man have been described in animals. Mayer (1921) described *Bartonella muris* the cause of infectious rat anaemia. Kikuth (1928) demonstrated *Bartonella canis* in the dog and Schilling (1928) recorded *B. muris musculi* in the mouse. In several wild rodents various other species have been observed.

A common symptom produced by these organisms is an anaemia characterised by degenerative and regenerative changes in the blood. These changes occur sometime after the appearance of the parasites, in a manner similar to that observed after infection with *Anaplasma*. In fact, between Anaplasmosis and Bartonellosis there appears to be some relationship. The morphology and staining properties of the causal organisms are very similar, the clinical syndrome in each case is practically identical and in both conditions splenectomy is followed by a reappearance of the parasites in the blood.

Closely related to *Bartonella* is the genus *Eperythrozoon*. *E. coccoides* was first described by Schilling (1928) in splenectomized mice. Shortly afterwards Dinger working independently proposed the name *Gyromorpha musculi* for the same organism observed in splenectomized mice in Amsterdam. These observations were compared by Mesnil, Brynoghe, Vassialidis, Elliot and Ford. The latter recorded their occurrence in *Arvicola arvalis* and *Mus minutus* in addition to *Mus musculi*. Schwetz (1933) reported their occurrence in field mice in the Belgian Congo.

Schilling, Dinger and other workers found that 2-4 days after splenectomy of infected mice small ring-shaped organisms 0.5μ - 1.0μ in diameter appeared in blood smears stained by Giemsa. Dinger described only ring forms but Kikuth in addition found rod-shaped and coccus forms. The parasites occur free in the plasma in addition to being found on the red cells. For example Dinger mentions that on the 2nd day after the appearance of parasites in splenectomized mice their number increases especially in the plasma where they may be 20-30 times as frequent as erythrocytes. On the 3rd and 4th day of infection large numbers can be seen lying on the red cells especially on the polychromatic erythrocytes. After a further interval of 24-48 hours the parasites may have disappeared but there is usually a periodic reappearance over a period of many weeks.

The course of *Eperythrozoon* infection in white mice differs markedly from that seen in field and wild mice. In striking contrast to the severe anaemia seen in infectious rat anaemia produced by *Bartonella muris*, white mice do not appear sick and any anaemia definitely is not severe. On the other hand, Kikuth in his work on wild mice observed severe clinical symptoms and even death in some of his animals. After experimental infection he observed anaemic changes characterised by erythro-phagocytosis and the appearance of numerous normoblasts. An extraordinary phenomenon was the extreme fluctuation in the number of parasites in this respect the behaviour closely resembling that of *Bartonella canis*. This periodic disappearance and reappearance of parasites might be observed 4 or 5 times. When the parasites were rare ring forms could be differentiated easily from rod and coccus forms but when the parasites were frequent *Bartonella* forms were found almost exclusively. This variation in the number of parasites together with the production of severe clinical symptoms anaemia and even death led Kikuth to believe he was working with a mixed infection of *Eperythrozoon coccoides* and *Bartonella muris musculi* but on subinoculation into splenectomized white mice these animals showed no clinical symptoms and only ring forms were encountered.

In spite of some difference in morphology, in location of the parasites, and in the clinical picture produced by infection with *Eperythrozoon* and *Bartonella* it must be concluded that the two parasites are closely related. There is no conclusive evidence for the acceptance of *Eperythrozoon* as a parasitic entity and not as a product of desquamation but the transmission from infected to susceptible white mice and rats, their reappearance after splenectomy, and the chemotherapeutical influence of neosalvarsan in sterilising the blood is significant.

OBSERVATIONS AT ONDERSTEEPOORT.

During the course of experimental work on Heartwater (*Rickettsia rummantium* infection) 10 c.c. of blood from a sheep at the height of the Heartwater reaction was injected into a splenectomized sheep. Four days later peculiar ring-shaped bodies $0.5\ \mu$ – $1.0\ \mu$ in diameter were observed in blood smears from this sheep stained with Giemsa. At first these bodies were regarded as stain deposit or artefacts but each of these possibilities was ruled out by taking meticulous care with the cleaning of slides and the preparation of the blood fibres, and by staining with filtered stain in the inverted position. The bodies which were to be seen on the red cells as well as free in the plasma, increased in number daily. On the fifth day, i.e., several days before the "virus" of Heartwater could be expected to be found in the peripheral circulation, blood was subinoculated into three normal sheep. The peculiar organisms were found in each of these sheep from the 7th day, and were carried on for several generations by repeated subinoculations. The original splenectomized donor succumbed to Heartwater on the 13th day after injection; none of the subsequent generations of sheep developed Heartwater to which disease their susceptibility was demonstrated later by immunity tests.

Smears were prepared daily from each of the subinoculated sheep and from these preparations stained with Giemsa it is possible to give an accurate description of the peculiar parasites. It is now apparent that these organisms had been observed previously on many occasions but their significance had not been appreciated fully.

DESCRIPTION.

The organisms may be found lying supracellularly on the erythrocytes but the greater number are to be found lying free between the cellular elements in the blood smears.

Position. (a) *The supracellular forms.*—The number of parasites lying on the erythrocytes varies within very wide limits, but usually the number of supracellular forms is directly proportionate to the intensity of the infection in the blood. A single organism may be present or alternately the entire surface of a red cell literally may be covered with parasites which may there be lying on top of one another. The most common occurrence is for the supracellular forms to be found in clusters of from 3 to 12 aggregated towards the centre of the cell, or at a point towards the periphery or along a portion of the border.

(b) *The extracellular or free forms.*—Invariably the free forms are the more numerous. As will be seen from the photographs at the end of the text, they are fairly evenly distributed in desquamated groups throughout the preparations.

Morphology.—Typically the organisms appear as delicate rings approximately 0.5 – $1.0\ \mu$ in diameter. Fixed with May-Grünwald and stained with Giemsa they take on a delicate pale purple colour. In the ring-forms about $4/5$ of the centre portion fails to take the stain and they appear to be flat, not spherical. They predominate both supra- and extracellularly but can but be studied in those

portions of a smear where clumps of the cells are widely separated. In addition to the ring forms there are frequently to be seen ovoid forms, irregular triangles with the angles rounded off, in addition to rod, dumbell, and comma shaped forms.

A point of interest is that it has been noted frequently that at one end of a smear ring forms predominate while towards the other end rod and comma shaped forms are more frequent. It is assumed that this distribution is purely mechanical and may be the result of injury during the process of drawing the film.

In some of the ring forms there may be noticed one, two or even three points which stain an appreciably darker colour. Their significance is quite obscure but they may stand in some relation to multiplication.

Symptomatology.—Up to the present the disease has only been studied in Merino sheep under stable conditions. As yet nothing can be said about the cause of infection in other breeds of sheep, or in animals subjected to adverse conditions in the field, where it is assumed that the symptoms would be more pronounced and where the disease may be of some economic importance. In addition nothing whatever is known of the natural mode of transmission.

After artificial infection with blood by the subcutaneous or intravenous route the period of incubation usually varies from 4–15 days, but it may be extended up to several weeks. The first symptom is fever, the temperature rising to about 105° F., though it has been observed to rise as high as 107° F. The febrile reaction may be continuous for 3 or 4 days, or it may be intermittent. Febrile exacerbations and remissions at intervals of a week or more are common or alternately there may be a complete absence of any hyperthermia.

Parasites make their appearance concurrently with the first rise in temperature or they may be seen only a few days later. They then multiply rapidly and within a week may be 25 to 100 times as numerous as the erythrocytes. From the limited number of animals examined it would appear that active multiplication of the parasites continues up to the time when the first signs of anaemia make their appearance in the smears. Then the number of parasites suddenly decreases and when the anaemia is at its worst comparatively few organisms are to be seen. When the condition of the blood tends to become normal again, usually after about 4 weeks, there may be a recrudescence of infection again. This irregular fluctuation in the degree of infection may continue for 6 weeks or longer.

While the fever curve is not very characteristic for the disease the anaemia is a constant and regular symptom. Clinically it may be demonstrated 5 to 8 days after the first appearance of parasites in the smears. As the condition progresses the visible mucous membranes become more and more pale until eventually they have the appearance of white porcelain. A slight icterus has been observed in a few instances. Examination of the blood shows a rapid drop in the red precipitate on centrifugation and the red blood count may drop as low as 1,500,000 within 10 days. At the same time there is a rise

in the leucocyte count up to about 20,000. This increase is due chiefly to an absolute and relative monocytoses and erythrophagocytosis is a common feature. With the development of anaemia the results of degenerative and regenerative processes make their appearance, namely anisocytosis, polychromasia, punctate basophilia, jolly bodies and nuclear rests in addition to normoblasts. Provided no relapse occurs the blood picture returns to normal in about 4 weeks.

Other clinical symptoms are those of fever and anaemia and vary with their severity. One notices dullness, inappetence, loss of condition and debility. The pulse is rapid and weak and the respirations accelerated.

A fatal termination has not been noted and the prognosis under stable conditions is good.

DISCUSSION.

Since the recognition of the above described organism as a parasitic entity, it has become apparent that certain febrile reactions and anaemic changes in the blood of sheep, which were inexplicable at the time, may now be accounted for. The ease with which the parasite may be transmitted by artificial means in the laboratory must serve as a warning to research workers particularly those engaged in haematological studies of other infectious diseases. Whether the disease is of any importance from an economic point of view in the field still remains to be determined but it appears not unlikely that the extreme and prolonged anaemia may be of importance in reducing the natural resistance of affected animals to other conditions which alone may not be fatal. This aspect of the problem together with the natural mode of transmission is receiving attention.

A consideration of the parasite, its morphology, location, staining reaction with Giemsa and the nature of the disease produced, has led the authors to believe that it is a hitherto undescribed species of the genus *Eperythrozoon* for which the name *Eperythrozoon oris* is proposed.

Classification.—*Eperythrozoon* bears an unmistakable resemblance to *Bartonella* and *Grahamella*. In many respects it appears to have a fairly close relationship with *Anaplasma* as well as was noted above. It is interesting to note the morphological differences between the type species of these four genera. *Anaplasma* consists of grains of chromatin, round oval or somewhat sharply irregular in shape. They are intracellular though occasionally they give the distinct impression of being epicellular and there are invariably only 1 or 2 (never more than 3 or 4) parasites in each erythrocyte. *Grahamella* consists of fairly regular rods which are intracellular and are always present in fairly large number (8–20) in the same cell. *Bartonella* is pleomorphic being represented by cocci, rods, rings and various irregular shapes. Their position is intracellular and they are always present in fairly large numbers. *Eperythrozoon* is also pleomorphic although the variety of shapes is probably not so great as in the case of *Bartonella*; ring and rod shapes predominate,

and the infection may be very heavy. The chief difference between these two genera is the situation of the parasites which in the case of *Eperythrozoon* is epicellular or extracellular.

As regards the result of infection with these different genera. *Anaplasma* in cattle causes a very severe disease characterised by extreme anaemia and the condition is frequently fatal; *Bartonella* in man is responsible for a serious condition (Oroya fever and Verruga peruviana); *Eperythrozoon* appears to result in nothing more serious than the production of fever followed by a severe anaemia. *Grahamella* is non pathogenic.

In view of this obvious relationship between these four genera it is proposed tentatively that they should be included in the family *Anaplasmidæ*. This family which most protozoologists would presumably place in the order *Haemosporidia* could be defined as follows:—

Small blood parasites round, oval, rodshaped or irregular in shape whose average size is 0.5–1.0 μ . They lie either in or on the red cells or occur free in the plasma. They may be non-pathogenic or pathogenic, in which case they most frequently produce fever followed by a variable degree of anaemia. Transmission takes place probably in all instances by arthropods.

In passing it may be mentioned that *Rickettsia* has many features in common with the above group or organisms but its affinity for endothelial cells and its almost complete absence from the blood stream microscopically would seem to indicate a more distant relationship.

SUMMARY.

1. A brief review of a portion of the literature on *Bartonella*, *Grahamella* and *Eperythrozoon* is given.
2. A new species of blood parasite of sheep is described for which the name *Eperythrozoon ovis* is proposed.
3. The symptomatology of the disease produced by *Ep. ovis* is detailed.
4. The tentative proposal is made to unite the four genera *Anaplasma*, *Grahamella*, *Bartonella* and *Eperythrozoon* into one family the *Anaplasmidæ*.

LITERATURE.

Full lists of references to *Bartonella*, *Eperythrozoon*, and *Grahamella* will be found in the publication of Kikuth, 1932.

DINGER, J. G. (1926). Näheres über das Eperythrozoon Coccoides. *Zeit. Bakter. Orig.*, 113, pp. 503-509.

DA ROCHA-LIMA, H. (1926). Verruga peruviana. *Menses Handbuch der Tropenkrankheiten*, 3 Aufl., Bd. 3, pp. 355-396.

- KIKUTH, W. (1928). Ueber einen neuen Anaemieerreger. *Bartonella canis*, nov. spec. *Klin. Wschr.*, 1928, pp. 1729-1730.
- KIKUTH, W. (1932). Die Bartonellen und verwandte Parasiten bei Mensch und Tieren. *Ergebnisse der Hygiene Bakteriologie Immunitätsforschung und Experimentellen Therapie, Band XIII*, pp. 559-619.
- SCHILLING, V. (1928). Eperythrozoon coccoides, eine neue durch Splenektomie aktionierbare Dauerinfektion der weissen Maus. *Klin. Wschr.*, 1928, pp. 1853-1855.
- WENYON, C. M. (1926). *Protozoology*, Vol. 1 and 2.

ADDENDUM.

Neitz and Quinlan found a species of *Eperythrozoon* in splenectomized calves at Onderstepoort, which were known to be carriers of *Anaplasma centrale*. After the operation *Eperythrozoon* appeared in extremely large numbers, and also *Anaplasma centrale* were present. At present it can not be stated whether the *Eperythrozoon* of cattle is identical with *Eperythrozoon ovis* of sheep, but transmission experiments to clear up this point are being undertaken.

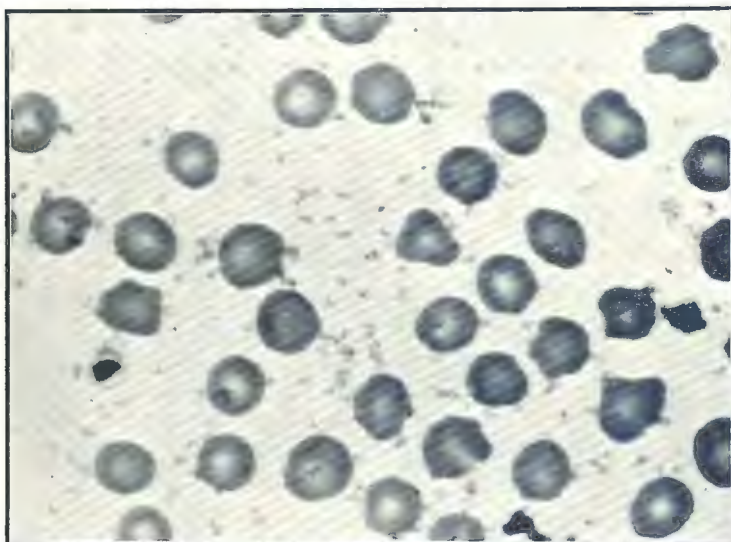


Fig. 1.—*Eperythrozoon ovis* of sheep. Numerous parasites lying free, 4th day of infection. Magnification 1,750 \times .

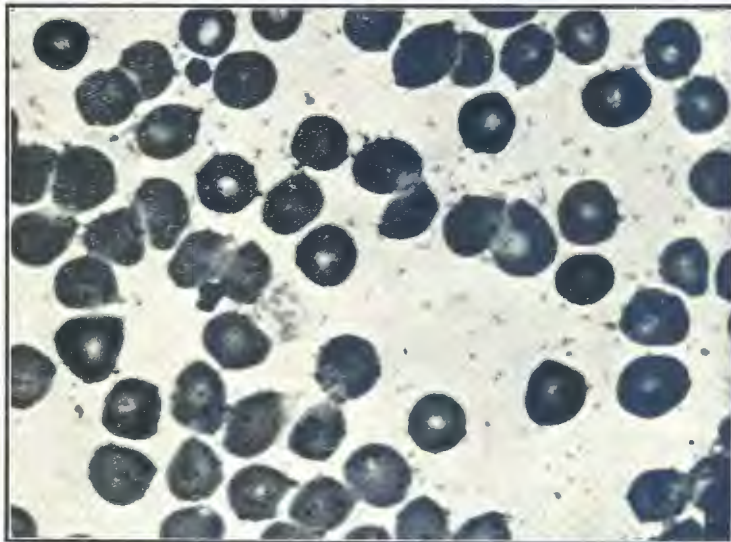


Fig. 2.—*Eperythrozoon ovis* of sheep. Some parasites are free and some supracellular. Magnification 1,500 \times .

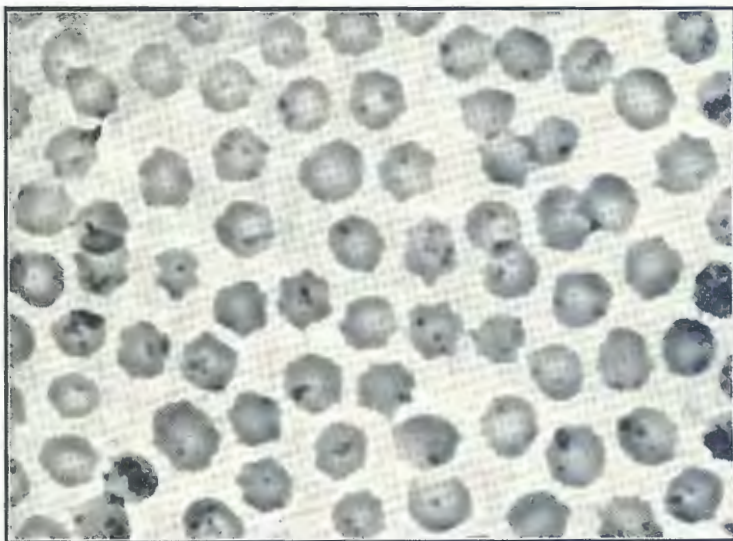


Fig. 3.—*Eperythrozoon ovis* of sheep. Parasites lying supracellular. Magnification 1,750.

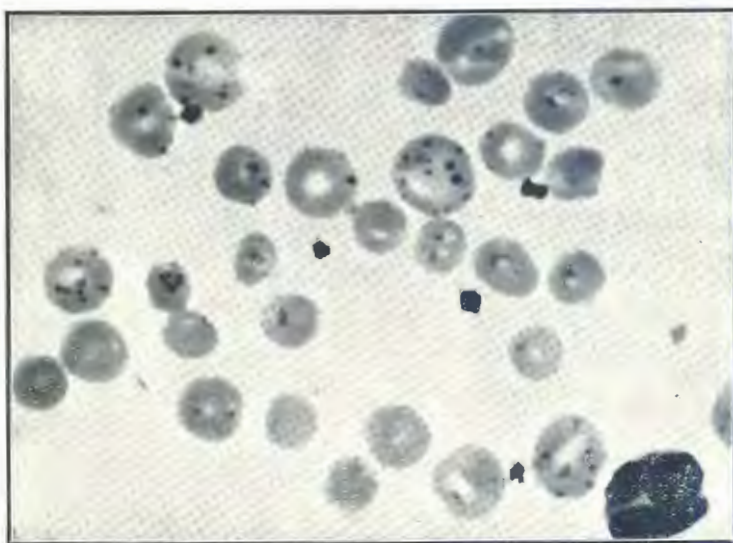


Fig. 4.—Anaemic changes due to *Eperythrozoon* infection. (Anisocytosis and punctate basophilia.) Magnification 1,500 \times .