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Eperythrozoonosis in Sheep.

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CONTENTS.

1. Introduction.

The presence of *Eperythrozoon ovis* in the blood of sheep at Onderstepoort was reported by Neitz, Alexander and du Toit in an address to the Biological Society of Pretoria, on March 15th, 1934, and was recorded in this journal the same year. Since the appearance of that original publication research into the problem of Eperythrozoonosis has progressed. The results obtained form the subject of this paper.

It will be seen, however, that there remains a considerable amount of work to be completed, but it is considered that the publication of an interim report is justified owing to the slow progress being made, chiefly due to the fact that 15-20 per cent. of available experimental sheep harbour a latent infection of the parasite.

Frequent references were made to the work on Oroya fever in man and *Bartonella* and *Eperythrozoon* infections in rats and mice since those studies have proved an invaluable guide to investigations into the analogous disease in sheep.

2. Definition.

Eperythrozoonosis of sheep is an infectious disease caused by *Eperythrozoon ovis*, a small supra- and inter-cellular blood parasite having a ring, rod, irregular or oval shape and belonging to the family *Anaplasmidae*. Initial invasion of the organism results in the production of irregular pyrexia, a variable degree of icterus, and anaemia characterized by the appearance of degenerative and regenerative elements in stained blood films. Relapses at varying intervals after recovery may occur. The mode of transmission is not known, but it would appear that the vector is a blood-sucking arthropod.

3. History.

It is difficult to ascertain to what extent unidentified infection of *Eperythrozoon ovis* has been a complicating factor in previous work on anaplasmosis, heartwater, bluetongue, trypanosomiasis, verminosis and other diseases in sheep. For instance, de Kock and Quinlan (1926) in their report on the results of splenectomy in sheep state " Another observation to be recorded in connection with the erythrocytes of some of the sheep is the appearance of a peculiar irregular reticular-like network. These varied in shape from long irregular threads to network-like masses resembling large piroplasms. No chromatin or typical cytoplasm could be identified in them ". In some sheep they observed these structures alone, in others they were associated with *Anaplasma ovis*, but the nature of these irregular reticular masses remained unexplained. It would appear now that an infection with Eperythrozoon had been encountered. Similar structures have been observed by other workers when examining stained blood films, but in each case they were dismissed as extraneous material, stain deposit or artefacts. Since the recognition and description of the parasite by Neitz, Alexander and du Toit in 1934, it has become apparent that certain unexplained anaemic changes in the absence of anaplasmosis or verminosis can now be accounted for.

Nothing is known of the rôle played by this parasite in the field. Farmers in South Africa describe an anaemic condition in sheep to which the name bleeksiekte (pallor disease) has been given, but the aetiology is quite obscure and it is not known to what extent verminosis and malnutrition in addition to *Ep. ovis* are contributory factors.

4. Occurrence.

Little is known of the distribution of this condition in South Africa since it has not been possible to carry out a methodical survey. The majority of the Merino sheep used for experimental purposes at Onderstepoort are purchased in the Karroo, chiefly in the vicinity of Middelburg and De Aar in the Cape Province. Examination of these animals has revealed the presence of individuals showing active as well as latent infections. Further, the presence of infection has been diagnosed in Blackhead Persian sheep introduced into the Pretoria district from the Northern Transvaal. Bearing these observations in mind, together with the normal exchange of sheep at sales, the widespread practice of moving flocks of sheep during the winter months from the highveld to the lowveld, and the necessity of trekking in search of grazing during periods of prolonged drought, the belief is justified that *Ep. ovis* is widely disseminated throughout South Africa.

The occurrence of *Ep. ovis* in sheep has been described by Donatien and Lestoquard (1935) in Algeria, by Delpy (1936) in Iran and by Lafenetre (1936) in France.

5. Aetiology.

(*a*) *Classification.*

The close resemblance between *Eperythrozoon* on the one hand and *Bartonella* and *Grahamella* on the other hand, together with the apparent relationship to *Anaplasma* has been mentioned by Kikuth and other investigators. Neitz, Alexander and du Toit (1934) suggested that these four genera should be included in the family *Anaplasmidae*, but the exact position of the parasite in any scheme of classification has not been decided upon finally.

The description given is that of the appearance of the parasite seen on examination of rapidly dried blood films fixed by May-Grunewald and stained with Giemsa. Stained by this technique the organisms take on a delicate pale purple to pinkish purple colour. Typically they are seen as delicate rings approximately 0·5-1 μ in diameter though occasionally they may be somewhat larger. In addition to ring forms it is common to encounter triangles with rounded angles, ovoid, comma, rod, dumb-bell and tennis racket forms.

It has been observed frequently that at one end of the smear ring forms predominate while towards the other end the number of rod and comma forms is in the majority. It is believed that this distribution is purely mechanical, being brought about during the process of drawing the blood film.

(*c*) *Localization.*

Although large numbers of organisms are to be found lying supra-cellularly on the erythrocytes in blood smears, the majority appear to be free between the cellular elements, but this distribution varies within very wide limits. Usually the number of supra-cellular forms is directly proportional to the intensity of the infection, and

it has been observed frequently that during the first 24 or 48 hours after the appearance of the parasites practically all the organisms are to be found lying on the erythrocytes; as the disease progresses the proportion of free forms increases.

(*a*) *Supra-cellular Forms.*—A single organism may be present, but on the other hand the entire surface of a red cell literally may be covered with parasites which seem to be lying one on top of the other. Most commonly the supra-cellular forms are to be found in clusters of 3-12 aggregated towards the centre of the cell, or at a point towards the periphery or actually along portion of the border. A fairly characteristic picture is to find several rod shapes strung together along the periphery of a cell in such a way as to form a partial or complete ring. In some cases a very fine fibre commencing from a cluster or circle of ring forms, may be seen drawn a variable distance across the cell like a veil.

(*b*) *The Extra-cellular or Free Forms.*—These usually predominate and are fairly evenly distributed throughout the preparation. It has been observed sometimes in thick smears where the erythrocytes are packed together that the interstices are filled with a homogeneous mass that stains in a manner similar to the parasites.

It is not known whether the distribution of *Ep. ovis* in stained smears is a true picture of the distribution of the parasites in the circulation. After centrifuging defibrinated or oxalated blood for 1 hour at 3,000 revolutions per minute the majority of Eperythrozoa could be demonstrated in the layer just below the leucocytes. This indicates that the union between the parasites and the host cells is rather loose so that it is possible that a large number of the extra-cellular organisms may simply have been detached during the process of smear preparation. This point of view is supported by the repeated observation that the proportion of extra-cellular to supra-cellular parasites varies considerably in different preparations made from the same animal at the same time.

It is possible that multiplication may take place by budding, since occasionally smaller forms may be seen lying in contact with those normal in size. In some of the ring forms there may be noticed 1, 2 or 3 points which stain in an appreciably darker colour. The significance of these points is quite obscure, but they may stand in some relation to multiplication.

All attempts to cultivate *Ep. ovis* on the usual laboratory media have been unsuccessful.

6. Transmission.

Nothing is known about the natural mode of transmission of *Ep. ovis* in sheep. In the *Eperythrozoon* infection of mice the louse (*Polyplax serrata*) has been found to be the vector. No ectoparasites were found on a few Merino sheep that were showing an active infection of *Ep. ovis* which had been contracted naturally.

The intravenous injection of emulsified keds (*Melophagus ovinus*) collected from known carriers failed to set up the disease in a susceptible splenectomized sheep.

Artificially infection may be transmitted from sheep to sheep by the subcutaneous or intravenous subinoculation of blood or emulsified organs.

7. Pathogenicity.

Up to the present the disease has been studied at Onderstepoort only in Merino sheep, so that nothing can be said of the relative susceptibility of, and the course of the disease in, the different breeds of sheep.

The susceptibility of different adult Merino sheep varies considerably as evidenced by variations in the degree of anaemia and icterus in different individuals after artificial infection. From the limited number of cases studied it would appear that there is no difference between the susceptibility of lambs and young sheep and that of adults.

Artificial infection of a susceptible splenectomized calf was successful, resulting in the production of severe anaemia and icterus. On the other hand, a splenectomized dog, together with rabbits and guinea-pigs, proved to be refractory.

8. Pathogenesis.

The Eperythrozoa parasitize the erythrocytes, but it is not clear how destruction is brought about. Varying with the intensity of infection a larger or smaller number of red cells is destroyed resulting in the production of anaemia. Examination of the blood has shown that there is a rapid drop in the red precipitate obtained on centrifugation and the red cell count may fall as low as 1,000,000 per c.c. within 10 days. The decrease in the number of erythrocytes commences at the time of the first appearance of parasites. Simultaneously there is a rise in the leucocytic count up to 20,000 per c.c. This increase is accounted for by an absolute and a relative monocytosis. Erythrophagocytosis is well marked. With the development of anaemia evidences of degenerative and regenerative processes are seen in stained smears, namely anisocytosis, polychromasia, punctate basophilia, reticulocytes, jolly bodies, nuclear rests and normoblasts. Haemoglobinurea has been observed in one case where the destruction of red cells proceeded with great rapidity. Icterus is generally present.

According to Graf (1935), who examined the blood changes associated with *Ep. ovis* infection from a chemical point of view, " Severe anaemia was noted, the haemoglobin decrease being up to 60 per cent. of the initial haemoglobin value, decreasing over a period of three weeks post-infection to the minimum. Regeneration is relatively slow; three weeks after reaching the minimum level only approximately 60 per cent. of the initial haemoglobin count is attained. Although the maximum erythrolysis takes place during the hyperrhexia visible haemoglobin-anaemia was never observed; no spectroscopic examination of the serum for haemoglobin was undertaken. Bilirubinaemia of the indirect van den Bergh type was present, generally also icterus. The red cell count shows an enormous decrease, from about 10×10^6 to 2×10^5—i.e. more striking

than the haemoglobin decline, pointing to a high colour: count ratio; The morphological blood changes are well marked. The protein of the whole blood falls to 10 per cent. of normal, whereas the non-protein-nitrogen fraction rises slightly during the acme of the reaction, chiefly due to an increased urea-nitrogen. The amino-acid-nitrogen, the uric acid nitrogen, the rest nitrogen and total creatinine nitrogen show no significant changes ''.

9. Symptoms.

The symptoms and course of the disease have been studied in Merino sheep maintained under stable conditions and in a small camp where the animals were exposed to adverse climatic conditions, but where they had easy access to food and water. The observations are detailed in tabular form in Table I. The reactions in the stabled sheep did not differ from those in the sheep maintained outside in the camp except that in the latter the temperatures were usually appreciably higher and the daily fluctuations were wider. No opinion can be expressed as to the nature and course of the disease in sheep maintained under South African farming conditions where adverse climatic conditions, periodic shortages of food and water and inter-current infections such as verminosis might be complicating factors. In addition observations on a few splenectomized sheep were recorded; these are tabulated in Table II. In every instance the disease was set up by artificial means.

The average period of incubation is 5-7 days. Sub-inoculation of blood from a sheep during that period of a primary reaction when the number of circulating parasites is most numerous tends to decrease the period of incubation while a corresponding lengthening is observed after sub-inoculation of an equal dose of blood from a premune sheep during the period of latent infection.

The first appearance of parasites has been observed as early as the second day, but may be delayed as long as twenty-six days after infection by either the subcutaneous or intravenous route. On an average they are first observed on approximately the fifth to seventh day. The parasites multiply rapidly and within a week may be 25 to 100 times as numerous as the erythrocytes. They are present in greatest number usually between the fifth and tenth day after their first appearance, but this time may vary from the third to the fourteenth day. The organisms may be demonstrated microscopically in the peripheral blood for a period of 6-42 days with an average period of fourteen days. It would appear that active multiplication continues up to the time when the first signs of anaemia make their appearance in the smears. Then the number suddenly decreases so that when the anaemia is most marked comparatively few or no organisms may be seen. When the condition of the blood tends to return to normal there may be a recrudescence of infection. In those cases where parasites could be demonstrated continuously for 35-40 days a graphic representation of the daily number would show the presence of several peak periods, i.e. there was a marked fluctuation in the number of parasites circulating. Disappearance of the parasites may be followed by reappearance after an interval of days, weeks or months during which they cannot be demonstrated

microscopically. In one instance (sheep 37175) careful examination of blood smears over a period of 547 days showed that subsequent to the primary reaction three relapses occurred after intervals of 11, 23 and 109 days during which the blood was free from parasites.

In the course of the disease usually there is a distinct febrile reaction. The incidence of fever may be the first symptom but it may develop only subsequent to the appearance of parasites in the blood. The temperature may rise as high as 107° F. but usually it does not exceed 105°. Fever may be continuous for three or four days or it may be intermittent. Febrile exacerbations and remissions at intervals of a week or more are common, but alternatively there may be a complete absence of hyperthermia. Irregular hyperrhexia has been observed during the period when relapses occur.

Anaemia is a characteristic and constant symptom. It may be demonstrated clinically about five to eight days after the first appearance of parasites, and may last for a month or more. As the condition progresses the visible mucous membranes become more and more pale until eventually they take on the appearance of white porcelain.

Clinical icterus persisting for a few days has been observed in several cases. It is of interest to note that although an icteric condition of the mucous membranes may not be apparent yet the serum from such animals shows a varying degree of discoloration which may be demonstrated for a week or more. Only when the serum is a dark yellow colour does jaundice become apparent in a clinical examination of the living animal.

For the rest the symptoms are those associated with fever and anaemia, namely dullness, inappetence, loss of condition and debility, rapid weak pulse and accelerated panting respirations. One case showing haemoglobinuria has been encountered.

In splenectomized sheep the course of the disease did not differ appreciably from that described in non-splenectomized sheep above. Possibly the period of incubation was somewhat shorter and the rate of multiplication of the parasites rather more rapid, but this was hardly significant.

10. Prognosis.

Up to the present time mortality has been recorded once in the experimentally infected Merino sheep. No opinion can be expressed as to the possible termination after natural infection under adverse conditions in the field. Even though it would appear that mortality need not be feared, the disease if widespread must be of considerable economic importance because of the severe constitutional disturbance, the anaemia, debility and rapid loss of condition.

11. Pathological Anatomical Changes.

Since mortality only occurred once during the course of these experiments post-mortem examinations were carried out on sheep destroyed at various stages during the reaction. In general it may be stated that the lesions closely resemble those seen in anaplasmosis.

TABLE 1.

Observations in Artificially Infected Non-splenectomized Sheep.

PRIMARY REACTION.							RELAPSES DURING PERIOD OF OBSERVATION.				
D.O.B. No. of Sheep.	Infected from.	Total period of observation in days.	Incubation period in days	No. of days during which *Ep. ovis* was present.	Nature of infection.	Remarks.	No. of relapses.	Interval in days of parasite free period.	No. of days during which *Ep. ovis* was present.	Nature of infection.	Remarks.
35448	39466	63	2	15	6+	Severe Anaemia.	—	—	—	—	—
35449	39466	63	2	12	6+	,, ,,	—	—	—	—	—
40937	41030	18	4	6	6+	Severe Anaemia.	—	—	—	—	—
41053	41030	19	5	9	5+	Severe Anaemia.	—	—	—	—	—
41016	41199	83	5	9	5+	,, ,,	2	18	1	2+	Anaemia.
								7	15	4+	,,
41038	41424	34	5	11	5+	,, ,,	—	—	—	—	—
41585	41968	51	5	11	6+	,, ,,	—	—	—	—	—
41537	40968	51	5	13	5+	,, ,,	—	—	—	—	—
41581	40968	51	5	13	6+	,, ,,	—	—	—	—	—
41422	40951	25	5	13	5+	,, ,,	—	—	—	—	—
41097	41199	72	5	14	5+	,, ,,	—	—	—	—	—
41555	40968	51	5	15	5+	,, ,,	—	—	—	—	—
37978	37397	51	5	39	5+	,, ,,	—	—	—	—	—
37309	37385	86	5	40	5+	,, ,,	—	—	—	—	—
41510	40968	51	7	7	4+	Severe Anaemia.	—	—	—	—	—
41530	40968	51	7	10	5+	,, ,,	1	12	19	5+	Slight Anaemia.
36994	35798	56	7	11	6+	,, ,,	2	7	15	5+	Anaemia.
								4	4	3+	
41541	40968	51	7	19	5+	,, ,,	—	—	—	—	—
37385	32730	105	7	20	5+	,, ,,	—	—	—	—	—
41527	40968	51	7	23	4+	,, ,,	—	—	—	—	—
41572	40968	51	7	26	5+	,, ,,	—	—	—	—	—
37447	32730	52	7	28	6+	,, ,,	2	15	2	2+	Slight Anaemia.
								16	1	2+	,, ,,
37397	32730	105	7	36	6+	,, ,,	—	—	—	—	—
40127	39466	42	7	10	5+	,, ,,	—	—	—	—	—

TABLE I—(*contiued*).

	PRIMARY REACTION.						RELAPSES DURING PERIOD OF OBSERVATION.				
D.O.B. No. of Sheep.	Infected from.	Total period of observation in days.	Incubation period in days	No. of days during which *Ep. ovis* was present.	Nature of infection.	Remarks.	No. of relapses.	Interval in days of parasite free period.	No. of days during which *Ep. ovis* was present.	Nature of infection.	Remarks.
41532	40968	51	8	8	5+	,, ,,	1	22	2	2+	—
41520	40968	51	8	11	4+	,, ,,	—	—	—	—	—
41523	40968	51	8	11	5+	,, ,,	—	—	—	—	—
41563	40968	27	8	12	5+	,, ,,	—	—	—	—	—
35798	37874	48	8	13	5+	,, ,,	—	—	—	—	—
41588	40968	51	8	38	5+	,, ,,	—	—	—	—	—
37096	35096	131	9	11	6+	Severe Anaemia.	1	7	18	5+	—
41424	40851	26	9	7	5+	,, ,,	—	—	—	—	—
41513	40968	51	9	10	4+	,, ,,	2	23	1	+	—
								7	1	+	
41574	40968	51	10	8	3+	Severe Anaemia.	—	—	—	—	—
40951	41422	43	12	22	6+	,, ,,	—	—	—	—	—
41001	41424	34	13	14	5+	,, ,,	—	—	—	—	—
41538	40968	51	14	17	3+	,, ,,	—	—	—	—	—
41107	35449	40	15	19	5+	,, ,,	—	—	—	—	—
41054	41030	66	19	13	5+	,, ,,	—	—	—	—	—
37175	37429	574	19	26	6+	,, ,,	3	4	19	4+	Anaemia.
								23	14	5+	,,
								109	14	6+	,,
35781	35798	57	26	6	5+	,, ,,	—	—	—	—	—

NOTE.

N indicates negative for parasites.
2+ ,, parasites very rare.
3+ ,, parasites rare.
4+ ,, parasites frequent.
5+ ,, parasites very frequent.
6+ ,, parasites extremely frequent.

TABLE II.

Observations in Artificially Infected Susceptible Splenectomized Sheep.

PRIMARY REACTION.							RELAPSES DURING PERIOD OF OBSERVATION.				
D.O.B. No. of Sheep.	Infected from	Total period of observation in days.	Incubation period in days	No. of days during which *Ep. ovis* was present.	Nature of infection.	Remarks.	No. of relapses.	Interval in days of parasite free period.	No. of days during which *Ep. ovis* was present.	Nature of infection.	Remarks.
32730	—	13	4	10	6+	Severe anaemia. Died from heart-water.	—	—	—	—	—
32704	36994	533	3	25	6+	Severe anaemia.	2	7 67	10 2	5+ 2+	Anaemia. No anaemia.
32729	Pooled blood from 8 sheep.	365	18	10	5+	Severe anaemia.	—	14	18	2+	Anaemia.

In an animal destroyed after parasites had been observed for three days in blood smears, no macroscopic lesions were visible.

In sheep destroyed later in the reaction there was observed a marked anaemia sometimes associated with a variable degree of icterus not only of the subcutaneous and subserous tissues but also of the liver, lung and kidneys. The liver showed the presence of fatty degeneration. The gall bladder usually was distended with dark green viscous bile. There may be a marked hydropericardium together with a gelatinous infiltration of the subcutaneous tissues along the ventral portion of the neck. Regularly marked tumor splenis was present, this organ on section showing a reddish brown soft but not fluid pulp and extensive hyperplasia of the Malpighian bodies. Apparently this tumor splenis persists for some considerable time after recovery, in fact it still persisted in two sheep slaughtered six weeks after recovery.

Da Rocha-Lima (1926) in illustrations of endothelial cells from cases of Oroya fever in man, indicates the presence of granular inclusions. Strong and his co-workers have expressed the opinion that at some stage of the life cycle of *Bartonella bacilliformis* the parasites multiply in the endothelial cells. In the case of *Ep. ovis* infection of sheep no intracellular inclusions were found in endothelial scrapings from the jugular veins or in impression preparations of the brain.

12. Diagnosis.

Since there is no clinical symptom which may be considered pathognomic for the disease, diagnosis is dependent upon the microscopic demonstration of parasites in blood smears. In cases of severe anaemia, irrespective of association with icterus or not, eperythrozoonosis must be taken into consideration, the diagnosis being complicated by the observation that during the acute anaemic phase of the disease parasites frequently are absent from the peripheral blood stream. This necessitates either daily examination of smears over a period of days or even weeks in the hope that a recrudescence of parasites may occur or, alternatively, subinoculation of blood into susceptible sheep which in turn must be kept under observation for lengthy periods.

Should it be determined at some future date that the condition is responsible for mortality in the field, post-mortem examination alone would hardly be of assistance in arriving at a diagnosis, since the pathological anatomical changes are common to a number of other conditions or combination of conditions more frequently encountered.

13. Differential Diagnosis.

Any condition or combination of conditions which results in the production of anaemia and icterus may lead to confusion. Thus verminosis, particularly haemonchosis and gaigeriasis, pernicious anaemia of sheep caused by a virus described in Algeria, anasplasmosis, babesiosis, trypanosomiasis, enzootic and bacterial icterus, must not be lost sight of. In all such cases a final diagnosis usually will

only be reached after adequate microscopic blood examination. This necessitates directing attention to the fact that undoubtedly *Eperythrozoon* has been seen frequently by many investigators over a number of years and was not recognized as a parasitic organism. Invariably the parasites were discussed as dust, dirt, stain deposit or artefacts in the preparations; a knowledge of the morphology together with a little experience and the exercise of scrupulous care in every aspect of the preparation and staining of blood smears will obviate repetition of these errors.

14. Treatment.

(*a*) *General.*

The nature and symptoms of the disease are such that in the first instance attention should be paid to the feeding and general hygiene of the animals. Adequate feed of high nutritive value should be made easily accessible and the animals should be housed under conditions which will minimize the effect of adverse climatic conditions. The administration of iron and copper salts to promote blood regeneration is indicated but, for the rest, specific drug therapy is the most urgent need.

(*b*) *Specific Chemotherapy.*

From the point of view of specific chemotherapy the arsenic and antimony-arsenic compounds have proved to be of the greatest value, but the practical value of specific therapy in the absence of effective measures to prevent re-infection is not quite clear. In this respect a problem analagous to that experienced in the treatment of trypanosomiasis is encountered, namely that animals " sterilized " by the use of specific drugs subsequently become fully susceptible and are liable to re-infection in an acute form; in those cases where " sterilization " is not brought about, but where the drug has had a beneficial influence on the course of the disease, a relapse in an acute form may develop at any time.

(i) *Neosalvarsan.*—The specific action of arsenical compounds such as neosalvarsan, arsalyt and tryparsamide on *Bartonella* infection of rats has been described by Mayer, Borchardt and Kikuth (1927), high dilutions of the compounds being able to bring about disappearance of the parasites within 24 hours. Kikuth (1932) and subsequently other workers were able to show that arsenobenzols also had a direct action on *Bartonella canis* infection of dogs. Small doses of neosalvarsan resulted in a temporary disappearance of parasites whereas doses of 15 mgm. per kilo body weight produced complete sterilization. The authors state that this therapy must be regarded as a " therapia sterilisans magna " in the sense of Paul Ehrlich. Bruynoghe and Vassiliadis (1929) were prompted by the close relationship between *Bartonella* and *Eperythrozoon* to investigate the effect of neosalvarsan on the latter parasite; beneficial results were obtained immediately.

During the course of transmission experiments with *Eperythrozoon ovis* infection some of the sheep used were found to be insusceptible. This was a complicating factor of importance in the

TABLE III.

D.O.B. No. of sheep.	Infected from, Date; Dose 10 c.c. blood i.v.	Incubation period in days.	Before treatment parasites were present for.	Dose of neosalvarsan per Kg. body weight.	Nature of injection before treatment.	Nature of infection at different intervals after treatment.											After treatment smears were examined for.	Remarks.	Interval in days between treatment and reappearance of parasites.	Parasites present for.	Remarks.
						¼ hour.	½ hour.	¾ hour.	1 hour.	1½ hour.	2 hours.	3 hours.	4 hours.	5 hours.	6 hours.	24 hours.					
40724	35548 3/8/34	6	5 days	5 mg.	4+	—	—	—	4+	3+	3+	3+	3+	3+	3+	N	16 days.....	No anaemic changes developed.....	No parasites seen.....	—	—
40444	35548 3/8/34	4	7 days	5 mg.	4+	—	—	—	4+	4+	4+	3+	3+	3+	2+	N	16 days.....	Marked anaemic changes developed.....	No parasites seen.....	—	—
40962	35548 3/8/34	5	6 days	5 mg.	4+	—	—	—	4+	4+	4+	4+	4+	4+	4+	3+	16 days.....	Slight anaemic changes. *Ep. ovis* demonstrated for 16 days after treatment	—	—	—
40997	35449 3/8/34	4	7 days	7·5 mg.	4+	—	—	—	4+	4+	4+	4+	4+	4+	4+	4+	16 days.....	Severe anaemic changes developed. *Ep. ovis* disappeared 48 hours after treatment	No parasites seen.....	—	—
41004	35549 3/8/34	6	5 days	7·5 mg.	4+	—	—	—	4+	4+	4+	4+	4+	2+	2+	2+	16 days.....	Severe anaemic changes developed. *Ep. ovis* disappeared 48 hours after treatment	No parasites seen.....	—	—
40368	39466 6/7/34	6	8 days	15 mg.	4+	—	—	—	2+	N	N	N	N	N	N	N	45 days.....	Anaemic changes developed.....	21 days.....	13 days.	No anaemic changes were observed as result of the relapse. *Ep. ovis* frequent.
39853	39466 26/7/34	6	8 days	15 mg.	4+	—	—	—	2+	N	N	N	N	N	N	N	45 days.....	Anaemic changes developed.....	20 days.....	7 days..	No anaemic changes were observed as result of the relapse. *Ep. ovis* frequent.
41055	39853 28/8/34	6	4 days	30 mg.	5+	3+	N	N	N	N	N	N	N	N	N	N	50 days.....	No anaemic changes developed.....	37 days.....	11 days.	Slight anaemic changes were observed. *Ep. ovis* frequent.
41085	39853 28/8/34	3	7 days	30 mg.	5+	5+	N	N	N	N	N	N	N	N	N	N	50 days.....	No anaemic changes developed.....	36 days.....	11 days.	Slight anaemic changes developed as result of relapse. *Ep. ovis* frequent.
41105	37847 37855 28/8/34	4	6 days	30 mg.	5+	4+	4+	3+	3+	2+	N	N	N	N	N	N	3 days.....	Sheep died from heartwater.....	—	—	—
41544	40951 19/10/34	6	5 days	45 mg.	5+	N	N	N	N	N	N	N	N	N	N	N	45 days.....	Anaemic changes developed.....	29 days.....	4 days..	No anaemia developed as result of relapse. *Ep. ovis* not frequent.
41027	39466 26/7/34	7	Control	Not treated	*Ep. ovis* was very frequent and could be demonstrated for a period of 11 days. Severe degenerative and regenerative changes developed in the blood.																
35499	39466 21/7/34	2	Control	Not treated	*Ep. ovis* was very frequent and could be demonstrated for a period of 12 days. Severe degenerative and regenerative changes developed in the blood.																
35448	39466 21/7/34	2	Control	Not treated	*Ep. ovis* was very frequent and could be demonstrated for a period of 15 days. Severe degenerative and regenerative changes developed in the blood.																

TABLE IV.

D.O.B. No. of Sheep.	Injected from. Date. Dose 5 c.c. i.v.	Incubation period in days.	Before treatment parasites were present for.	Dose of Std. 386 B per Kg. body weight.	Nature of infection before treatment.	Nature of infection at different intervals after treatment.									Observations after Treatment.			Immunity Test.			
						¼ hour.	½ hour.	¾ hour.	1 hour.	2 hours.	3 hours.	4 hours.	5 hours.	24 hours.	Remarks.	Interval between treatment and reappearance of parasites.	Parasites present for.	Interval between treatment and immunity test.	Reappearance of parasites.	Parasites present for.	Remarks.
41024	41839 29/1/35	4	5 days	5 mg.	5+	5+	5+	4+	2+	+	+	+	—	—	Slight anaemia.....	35 days.....	7 days.....	110 days....	—	—	No reaction.
41807	,,	6	3 days	5 mg.	4+	4+	4+	2+	+	—	—	—	—	—	Slight anaemia.....	35 days.....	9 days.....	110 days....	17 days.....	7 days.....	Slight anaemia.
41819	,,	4	5 days	5 mg.	5+	5+	5+	4+	3+	—	—	—	—	—	Slight anaemia.....	—	—	110 days....	—	—	No reaction.
41835	41839 29/1/35	4	5 days	10 mg.	4+	4+	+	—	—	—	—	—	—	—	Slight anaemia.....	—	—	110 days....	17 days.....	29 days.....	Marked anaemia.
41836	,,	4	5 days	10 mg.	4+	2+	—	—	—	—	—	—	—	—	Slight anaemia.....	—	—	110 days....	17 days.....	10 days.....	Marked anaemia.
41858	41839 29/1/35	4	5 days	20 mg.	4+	2+	—	—	—	—	—	—	—	—	No anaemia.....	—	—	110 days....	17 days.....	29 days.....	Marked anaemia.
41861	,,	4	5 days	20 mg.	5+	3+	—	—	—	—	—	—	—	—	Slight anaemia.....	—	—	110 days....	18 days.. ..	14 days.....	Marked anaemia.
41875	41839 29/1/35	4	5 days	30 mg.	5+	+	—	—	—	—	—	—	—	—	No anaemia.....	—	—	110 days....	17 days.....	15 days.....	Marked anaemia.
41879	,,	6	3 days	30 mg.	3+	+	—	—	—	—	—	—	—	—	No anaemia.....	—	—	110 days....	—	—	No reaction.
41839	Control	Untreated. Parasites present for 12 days. Severe anaemia developed.																			

work so that it was decided, in view of the observations quoted above, to determine the effect of the administration of neosalvarsan. For the work (Exp. S. 5414) sheep were selected whose previous history showed that they had not received any injections of fresh blood, in other words, it was hoped that only susceptible sheep would be selected. These animals were infected experimentally by the intravenous subinoculation of blood from a sheep in the acute stage of the disease. When parasites made their appearance in the blood stream in large numbers, doses of neosalvarsan varying from 5-45 mg. per kilo were injected intravenously. After administration of the drug, smears were examined at half hourly or hourly intervals for six hours and then daily examination was continued. As controls three sheep remained untreated.

The results are summarized in tabular form. (Table III.)

Results.—1. *Dose* 5 *mg. Neosalvarsan per Kilo Body Weight.*—From two out of three sheep parasites had disappeared after twenty-four hours: no indications of anaemia were observed, and no parasites reappeared for sixteen days, at which time smear examination was discontinued. In the case of the third sheep parasites persisted for sixteen days after treatment and slight anaemia developed.

2. *Dose* 7·5 *mg. per Kilo.*—Two sheep were treated. In both cases parasites persisted for twenty-four hours, but had disappeared after forty-eight hours and did not reappear for sixteen days, when examination was discontinued. Both animals developed severe anaemia.

3. *Dose* 15 *mg. per Kilo.*—Two sheep. In both cases the number of parasites had markedly decreased one hour after treatment, and had disappeared after two hours. Slight anaemia developed. The blood remained free from parasites for twenty and twenty-one days respectively when they reappeared. In spite of this relapse no anaemic changes developed.

4. *Dose* 30 *mg. per Kilo.*—Three sheep. In the case of two sheep, parasites were no longer demonstrable half an hour after treatment, and no anaemic changes developed; thirty-six and thirty-seven days later respectively parasites reappeared, persisted for eleven days and resulted in the production of slight anaemia. In the case of the third sheep the number of parasites in the blood gradually decreased up to the second hour after treatment by which time they had disappeared. No further observations could be carried out as the animal died from an intercurrent infection of heartwater three days later.

5. *Dose* 45 *mg. per Kilo.*—One sheep. Immediately after injection of the neosalvarsan parasites were present in large numbers, but they had disappeared within fifteen minutes. Slight anaemia developed. After an interval of twenty-nine days parasites reappeared and persisted for four days, but no evidence of anaemia was found in the smears.

6. *Controls Untreated.*—Three sheep. *Ep. ovis* could be demonstrated for eleven, twelve and fifteen days respectively and the anaemia produced was much more marked than in any of the treated sheep.

Conclusions.—From the results detailed above it may be concluded that in sheep a single intravenous injection of neosalvarsan in a dose of 5-7·5 mg. per kilo body weight has a well-defined parasiticidal action upon *Ep. ovis* resulting in disappearance of the parasites from the blood stream within forty-eight hours. As the dose is increased, the rapid specific action becomes more marked, the destructive action being roughly proportional to the dose, so that when 45 mg. per kilo is administered all parasites are eliminated from the blood stream within fifteen minutes. On the other hand, even a massive dose of 45 mg. per kilo is not a sterilising dose, since parasites reappeared approximately four weeks after treatment.

Discussion.—In practice the use of neosalvarsan is indicated as a specific for the treatment of Eperythrozoonosis, since even in small doses it has a marked beneficial effect upon the course of the disease, but it must be borne in mind that even a massive dose will not result in complete sterilization. This finding is in striking contrast with the results obtained in cases of *Bartonella canis* infection of dogs, where one-third of the maximum dose used in sheep, namely 15 mg. per kilo, was found to be a sterilizing dose. No opinion can be expressed as to the possible effect of repeated intravenous injections of the drug.

(ii) *Arseno-stibio Preparation Std.* 386 *B.*—In the treatment of *Bartonella muris* infection, Yoshiwara (1931) found the chemotherapeutic index of the antimony preparation stibosan to be 1:8, and Mayer, Borchardt and Kikuth (1927) demonstrated that of neosalvarsan to be 1:72. Uhlenhuth and Seiffert (1931) reported the remarkable properties of the antimony-arsenic compounds of Std. 283 and Std. 246, which have a chemotherapeutic index of 1:400. Dr. Hans Schmidt, who was responsible for the preparation of these two drugs, subsequently evolved a third, namely arseno-stibio compound 386 B which Kikuth (1932) and Uhlenhuth and Seiffert (1933) found to have the extremely high index of 1:3,500. These authors found that 18-24 days after treatment *Bartonella muris* reappeared in the blood stream in the majority of cases, but they were unable to determine whether this was the result of reinfection or was in the nature of a relapse, since, under the conditions of the experiments, natural transmission could not be excluded.

Bearing in mind the specific action of neosalvarsan on both *Bartonella muris* and *Ep. ovis* infections it was decided to investigate the action of the drug with the highest chemo-therapeutic index, namely arseno-stibio preparation Std. 368 B, in sheep. This experiment (S. 5572) was planned on lines similar to those of the analogous work on neosalvarsan, the results being summarized in tabular form in Table IV.

Results. 1. *Dose 5 mg. per Kilo Body Weight.*—Three sheep. From two sheep the parasites had disappeared two hours, from one sheep five hours after treatment, and in each case slight anaemia only developed. Two of the sheep showed a relapse thirty-five days later, the parasites persisting for seven and nine days respectively; no parasites were observed in the blood of the third sheep for a period of one hundred and ten days, when an immunity test was applied to all three by the subinoculation of infective blood. As a result of this immunity test the one sheep which did not show a relapse did not react, but one of the two sheep which did relapse proved immune, while in the other parasites appeared after seventeen days and persisted for seven days. After the immunity test daily smear examination was discontinued for forty-six days.

2. *Dose 10 mg. per Kilo.*—(Two sheep.) Parasites had disappeared from the blood of both animals in thirty to forty-five minutes respectively, and only a slight degree of anaemia developed. Over a period of one hundred and ten days no parasites were observed in either sheep, but then on immunity test reinfection became apparent on the seventeenth day, parasites persisted for ten and twenty-nine days respectively being accompanied by severe anaemia.

3. *Dose 20 mg. per Kilo.*—(Two sheep.) The results from the use of this concentration of the drug were practically identical with those obtained from the use of 10 mg. per kilo.

4. *Dose 30 mg. per Kilo.*—(Two sheep.) There was a marked decrease in the number of parasites fifteen minutes after treatment, and all had disappeared within thirty minutes in both cases. On immunity test after a lapse of one hundred and ten days, during which time the blood remained *Eperythrozoon* free, one sheep became reinfected after an incubation period of seventeen days the parasites persisting for fifteen days and producing severe anaemia, while the other was found to be immune.

Conclusions.—From the above results it is concluded that arseno-stibio preparation Std. 386 B. is indicated as a specific in the treatment of eperythrozoonosis in sheep. Used in the small dose of 5 mg. per kilo body weight, it may be relied upon to eliminate the parasites from the blood with great rapidity and to have a beneficial effect upon the subsequent course of the disease; only in a percentage of cases will complete sterilization be the outcome. The number of sheep used to test larger doses of the drug is too small to permit any generalization. It is significant, however, that 10 and 20 mg. per kilo resulted in sterilization in each of two groups of two sheep, so that failure of one animal, which received 30 mg. per kilo, to react on immunity test is inexplicable.

TABLE V.

Splenectomy of Ep. ovis *Carriers.*

D.O.B. No. of Sheep.	Date of splenect-omy.	Total period of observa-tion in days.	*Ep. ovis* appeared after.	No. of days during which *Ep. ovis* was present.	Nature of infection.	Remarks.	No. of relapses.	Interval in days of parasite free period.	No. of days during which *Ep. ovis* was present.	Nature of infection.	Remarks.
37429	8/2/35	448	6 days	12	6+	Severe anaemia	5	47 52 61 88 103	7 9 10 5 4	4+ 6+ 5+ 4+ 4+	Slight anaemia. Severe anaemia. Anaemia. Anaemia. Anaemia.
37862	8/2/35	150	6 days	11	5+	Severe anaemia	1	123	2	3+	Anaemia.

15. The Effect of Splenectomy.

After the removal of the spleen from two known carriers of *Ep. ovis* a reappearance of parasites in large numbers in the blood stream occurred on the sixth day. Severe anaemia and symptoms of a primary acute infection as seen in a susceptible animal then followed. Examination of daily blood smears from one sheep (37429) over a period of 448 days showed that during that period five separate relapses occurred, the last commencing fifteen months after splenectomy. This recrudescence of *Ep. ovis* after removal of the spleen from sheep harbouring a latent infection is further evidence of the significant rôle of the spleen in the mechanism of immunity to protozoan infections.

16. Immunity.

Many essential details on the immunity in this disease are lacking. This is chiefly due to the fact that the manner of natural transmission is not known and consequently in all experimental work the possibility of accidental infection cannot be eliminated with certainty. Nevertheless the available data from observations on splenectomized and non-splenectomized sheep together with the results obtained from the use of massive doses of specific chemotherapeutical drugs indicate that immunity must be regarded as a " labile infection " or " immunitas non sterilisans " which leads to an equilibrium between the parasite and the host.

No cases of auto-sterilization have been observed.

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