

STUDIES ON SPECIFIC OCULO-VASCULAR MYIASIS OF DOMESTIC ANIMALS (UITPEULOOG): II.— EXPERIMENTAL TRANSMISSION

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A survey of all aspects of the reports on uitpeuloog did not exclude the possibility of an infectious agent being the primary aetiological factor. It was, therefore, decided to carry out a series of transmission experiments in an endeavour to throw some light upon this point.

It is not intended to offer any excuse for the manner in which these experiments were carried out in the field, the many defects and omissions being only too evident. It must be pointed out, however, that the State Veterinarian who carried out this work single handed had his office, which is provided with the minimum of laboratory equipment, at Windhoek situated some 400 miles from the enzootic area. Telephonic communication with the infected area was non-existent. The only transport was by road, for the most part over mere tracks through the sand.

No technical assistance whatever was available. All the work had to be sandwiched between the normal activities of a State Veterinarian in charge of an area which is fifteen thousand square miles in extent. This explains the fact that it was not possible to avoid delays in the examination of blood smears of experimental animals that were taken regularly, and that a careful examination of some reacting animals was unduly delayed.

To carry out the actual work a primitive field laboratory was improvised under a tree, in what shelter was available. Laboratory equipment was limited to the barest minimum. Some experimental animals were obtained from the local farmers but others were transported by truck, together with their fodder, the 400 miles from Windhoek, and, on arrival, could not be maintained under even relatively insect-free conditions.

Notwithstanding the many practical difficulties it will be seen, that this study, incomplete as it may be, laid the foundation for the elucidation of the aetiology of the disease.

EXPERIMENT NO. 1

The object of this experiment was to determine whether a brain emulsion prepared from acute natural cases showing involvement of the central nervous system might contain an infectious agent transmissible to other animals.

Two sheep that had shown well marked nervous symptoms for three and four days respectively were destroyed. The brains were removed and an approximate 10 per cent emulsion made by maceration in physiological saline sterilized by boiling. After filtration through sterilized surgical gauze, and standing for a few minutes

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to allow the coarser particles to settle, the emulsion was injected subcutaneously (15 ml) and epidurally (2·5 ml) into each of two local goats and one local sheep, as well as intraperitoneally (2·5 ml) and subcutaneously (2·0 ml) into each of three guinea-pigs and intraperitoneally (0·25 ml) into each of 10 mice.

The animals were kept under observation for 19 days. Except for a slight keratitis and conjunctivitis noticed in the sheep on the third day only, there was no deviation from normal health. Two mice died from injuries.

It was concluded that the brain emulsion was not infective.

EXPERIMENT NO. 2

The objects of this experiment were to determine whether—

- (a) the blood of reacting sheep contains an infectious agent transmissible to either sheep, goats or white mice;
- (b) there is any difference in the susceptibility of sheep and goats born and bred in the enzootic area and those imported from outside the affected area;
- (c) any nutritional factor associated with the local grazing might either cause uitpeuloog or affect the susceptibility of sheep.

The available experimental animals were divided into the following groups:—

Group A.—Four sheep and one goat obtained within the enzootic area and maintained on normal grazing in the enzootic area.

Group B.—Three sheep introduced from outside the enzootic area maintained on a ration of imported lucerne hay within the enzootic area; two of these sheep were to be retained as uninoculated controls for the duration of the experiment, as sentinels for intercurrent natural infection.

Group C.—Four sheep from outside the enzootic area maintained on lucerne hay outside the enzootic area.

Fresh cases showing severe symptoms were selected as donors of blood. One sheep from Group B, Z. 91, being the third passage sheep in that particular series, served as the donor for Group C.

Fresh blood for subinoculation was collected in sterile 3·8 per cent sodium citrate solution to make a final concentration of 1 per cent and was used for injection before the lapse of half-an-hour. Alternately blood was transfused direct, but this procedure was not favoured because the dose of blood could not be determined.

Each recipient received between 100 and 325 ml of blood, the average being 200 ml.

Results

Group A

Two sheep showed a slight rise in temperature on the third and fifth day respectively. One sheep showed an inclination to grind its teeth but otherwise there was no deviation from normal health. No symptoms whatever of infection of the eyes were seen.

The five animals were kept under observation for 19 days when the experiment was terminated as negative. The sheep were not slaughtered for post-mortem examination nor were blood smears taken.

Group B

The first sheep (Z. 122) inoculated in this series showed a sharp rise in temperature (104·2 °F) on the morning of the second day, accompanied by severe symptoms of the unilateral ophthalmic form of uitpeuloog. The fever reaction persisted for 48 hours after which the temperature returned to normal at which level it was maintained with minor fluctuations.

During the course of the first day of the febrile reaction, blood from the reacting sheep (Z. 122) was subinoculated into one of the two sheep (Z. 94) which were to have been retained as controls. A febrile reaction (temp. 104·3 °F) commenced after 24 hours, reaching a peak of 106·4 °F the following day, after which the temperature returned to normal only to be followed by a second similar febrile reaction after an interval of six days. The only symptoms were grinding of the teeth, some listlessness and anorexia. No symptoms indicative of any form of uitpeuloog could be detected.

To obtain more sheep to carry on this series would have entailed a return journey by road of not less than 800 miles, a trip which, in the circumstances could scarcely have been completed in less than three days. Any delay might have prejudiced the maintenance of infection. Reluctantly it was decided to use the last remaining control sheep (Z. 91) for subinoculation from reacting sheep Z. 94. During the incubation period of the disease in Z. 91, sheep could be obtained in the non-enzootic area to start the Group C series of subinoculations.

The reaction produced in Z. 91 was practically identical to the diphasic reaction shown by the donor Z. 94, but again none of the typical symptoms of uitpeuloog shown by the original reactor Z. 122 were seen. It must be pointed out that owing to the pressure of extraneous duties postponement to a later date of the examination of blood smears taken at the time of sub-inoculation and during the febrile reaction was unavoidable.

The experimental animals, none of which died during this series, were slaughtered at various times after subinoculation. The most important macroscopic pathological findings were the following.

Sheep Z. 122 (slaughtered after 23 days).—Keratitis, myocarditis, thrombo-endarteritis of the pulmonary artery, thrombo-endophlebitis of the jugular vein, tumor splenis and slight oedema of the lungs.

Sheep Z. 94 (slaughtered after 12 days).—Turbidity of the aqueous humour, peri-orbital haemorrhages, multiple petechial haemorrhages of the atrioventricular valves, tumor splenis and mild oedema of the lungs.

Sheep Z. 91 (slaughtered after 23 days).—Mild keratitis of the left eye, thrombo-endarteritis of the pulmonary artery, petechial haemorrhages in the medulla oblongata, tumor splenis and slight pulmonary oedema.

No facilities were available for histological examinations but stained smears from the vascular lesions showed a very conspicuous oesinophilia.

Subsequent microscopical examination of stained blood smears from these sheep showed that during the febrile reaction, spirochaetes were numerous, but that they could not be detected in smears prepared from the animals at the time of subinoculation.

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Group C

Febrile reactions identical with those produced in the sheep in Group B were obtained, but no evidence of any involvement of the eyes could be detected.

In an endeavour to maintain the infection by serial passage, citrated blood was forwarded to Onderstepoort by air. On arrival this blood failed to produce any reaction in sheep.

Three sheep were slaughtered for post-mortem examination, 17 days, 24 days and 26 days, after subinoculation. The only constant findings were slight tumor splenis with hyperplasia of the malpighian bodies, slight pulmonary oedema, subendocardial haemorrhages and nephrosis. Conspicuous by its absence was any sign of involvement of the eyes, or the thrombo-phlebitis and thrombo-arteritis so prominent in the sheep of Group B. Two sheep showed lesions of arteriosclerosis but this is believed to be of no significance since similar lesions were found in a few sheep slaughtered for other purposes.

The death of the fourth recipient three days after subinoculation terminated this experiment.

Subsequent examination of stained smears showed the presence of spirochaetes which were not found in smears prepared from the sheep at the time of subinoculation.

Small laboratory animals

No reaction nor pathological lesion was produced in white mice which received citrated blood by the subcutaneous or intraperitoneal route.

Conclusions and Comment

From this series of experiments it is believed that the following tentative conclusions are justified.

1. The blood of a sheep in the early or acute phase of the naturally contracted disease, contains an agent capable of setting up the disease when subinoculated in a large dose (Group B).

2. It is possible to maintain the disease by subinoculation of massive doses of blood, in the present series for three serial passages (Group B). The validity of the above two conclusions is open to doubt because the experimental animals were maintained under conditions, which did not exclude the possibility of accidental natural infection. However, the probability of coincidental infection on three occasions appears to be remote.

3. Sheep introduced from outside the enzootic area and fed a maintenance ration of lucerne hay, are susceptible (Group B).

4. Sheep born and bred in the enzootic area and maintained on the normal grazing are refractory to artificial infection (Group A). The validity of this conclusion is open to serious doubt and requires confirmation from the results of further critical experiments on larger numbers of animals in which there is positive evidence that the blood of the donor was infective at the time of subinoculation.

5. All reacting sheep showed the presence of large numbers of spirochaetes in blood smears made during the febrile reaction following inoculation. These spirochaetes, identified as *S. theileri*, were not present in smears made at the time of injection. At this stage no definite conclusion can be drawn as to the possible role of *S. theileri* in the aetiology of uitpeuloog.

6. Only those reacting sheep which showed involvement of one or both eyes, irrespective of the severity of the lesions showed on subsequent post-mortem examination macroscopic lesions of thrombo-phlebitis and thrombo-arteritis. It is worthy of note that these cardio-vascular lesions were more pronounced in those animals slaughtered for examination the longest after infection.

7. In spite of close contact between reacting and proved susceptible sheep, there was no evidence of the infection being contagious.

8. Infection was not transmitted to mice or guinea-pigs.

Additional experimental investigations

The following season natural cases of uitpeuloog were encountered and blood transfusions from one febrile case were again carried out in the non-enzootic area in sheep fed on lucerne hay. No difficulty was experienced in initiating and maintaining by serial passage the now well known febrile reaction accompanied by listlessness, anorexia and occasional grinding of the teeth. Even 10 ml of infective blood was used repeatedly with success. In one series, the febrile reaction was transmitted for 28 successive passages when it was abandoned voluntarily. There was no transmission to in-contact sheep. All reacting sheep showed variable degrees of spirochaete infection but not a single case of the ophthalmic form of uitpeuloog was produced, nor were lesions found in the cardio-vascular system of sheep examined post-mortem.

In an attempt to throw some light upon the pathogenicity of the spirochaetes, three separate experiments were carried out.

1. Citrated blood was centrifuged in an electrical centrifuge immediately after collection and the supernate passed under positive pressure through a Chamberlain B filter. The filtrate (45 ml) was injected intravenously into a sheep. No reaction was produced and on subsequent challenge with unfiltered blood (100 ml) after 26 days, the sheep showed the normal febrile reaction.

It was concluded that removal of the spirochaetes removed the infective agent.

2. Fresh infective blood (50 ml) was injected intravenously into a susceptible sheep simultaneously with 1,200,000 units of penicillin. Penicillin treatment (600,000 units) was repeated daily for three days.

Although the sheep developed listlessness and anorexia no febrile reaction was produced. Subsequent challenge with proved infective blood after an interval of 22 days produced no reaction.

3. Adult sheep were splenectomized and subsequently treated with infective blood. The anticipated febrile reaction was produced without the development of any eye lesions, but the general clinical picture was complicated by the development of theileriasis and anaplasmosis.

It was concluded that the *Spirochaeta theileri* infection is a transmissible disease to be regarded as an intercurrent complicating entity separate and distinct from uitpeuloog and responding to penicillin therapy.

EXPERIMENT NO. 3

The object of this experiment was to determine whether the blood of the blue wildebeest (*Gorgon taurinus taurinus* Burchell) contains an infective agent transmissible to sheep and goats and capable of producing uitpeuloog.

The appearance of migrating blue wildebeest afforded an opportunity of carrying out transmission experiments from this species of game.

As recipients there were available one sheep and one goat from locally bred stock and one sheep which had been introduced from a non-enzootic area. The two local animals were maintained on normal grazing and the imported sheep on imported lucerne hay within the enzootic area. They were not kept under insect-free conditions.

As donors three apparently normal wildebeest were shot at various times. As much blood as possible was collected in citrate from each donor for immediate subinoculation into an experimental animal. The smallest dose was 80 ml, the largest 300 ml intravenously.

Result

The goat at no time showed any deviation from normal health and in due course was discharged from the experiment.

The locally bred sheep developed a mild conjunctivitis of short duration from the third day and a temperature of 105°F was registered on the seventh day. Unfortunately, owing to the pressure of other duties this animal was not slaughtered for pathological examination.

The imported sheep showed a well defined bilateral exophthalmos after 36 hours with clearly visible intra-ocular haemorrhages. A febrile reaction (104.1°F) developed on the fifth day and subsided the following day.

After an interval of 21 days this sheep was slaughtered, the pathological findings being turbidity of the aqueous humour, thrombo-arteritis of the pulmonary artery, disseminated focal endocarditis, tumor splenis and a small area of yellow pigmentation of the cerebrum.

Unfortunately the blood smears collected were mislaid and could not be examined.

Conclusion

A sheep which received by intravenous injection a large dose of fresh citrated blood from an apparently healthy wildebeest developed the exophthalmos characteristic of uitpeuloog and on post-mortem examination showed involvement of the intima of the vascular system in addition to the eye lesions. The reaction commenced some 36 hours after subinoculation and it is believed that it would be stretching coincidence beyond the limits of probability to suggest that accidental natural infection had again taken place. Therefore, it is concluded that the disease is transmissible by subinoculation of fresh blood and that the blue wildebeest, in accordance with popular belief, is a link in the aetiological chain.

No conclusion may be drawn as to the relative susceptibility of imported or locally bred sheep or of the goat because a separate blood donor was used for each subinoculation.

DISCUSSION

From this series of experiments, admittedly incomplete and inconclusive, it is suggested that two highly significant conclusions may be drawn.

Associated with the ophthalmic syndrome is a well defined thrombo-endophlebitis and arteritis, not only of the vessels adjacent to the eye, but as far distant as the heart. The incidence of cardio-vascular lesions are not correlated with the severity of the eye lesions and, in sheep, should be regarded as the pathognomonic lesion upon which to base a diagnosis.

The disease is transmissible from reacting sheep in the early acute phase of infection by subinoculation of massive doses of fresh blood. A single successful transmission from an apparently healthy blue wildebeest is recorded.

The role of *Spirochaeta theileri* in the aetiology has not been defined clearly. In view of the fact that all but three sheep found to be reacting to spirochaete infection failed to show either lesions of the eye or the characteristic cardiovascular involvement, coupled with the observation that these spirochaetes have been encountered in numerous localities in which no case of uitpeuloog has been found, points to spirochaetosis being an incidental intercurrent infection unconnected with the specific disease. The identity of the specific disease-producing entity, therefore, remains obscure. However, the results of these experiments served as a stimulus for the further investigations reported in the third of this series of publications (Basson 1962).

No definite conclusion can be drawn as to the effect of local environmental factors, including the nutritional state, on the susceptibility of sheep to infection, but it is believed that at most they are of little importance.

SUMMARY

It has been shown that thrombo-phlebitis and thrombo-arteritis with prominent eosinophilia are constant lesions pathognomonic for uitpeuloog. The incidence of these lesions is not correlated with the severity of the eye lesions which previously were regarded as the pathognomonic symptom.

Uitpeuloog was transmitted by the subinoculation of large doses of fresh blood from natural cases in the acute phase of the disease. In one instance the infection was maintained by subinoculation in series for three passages, when it was lost.

A successful transmission with blood from an apparently healthy wildebeest is recorded.

In a single experiment brain material was found to be non-infective.

A frequent transmissible intercurrent infection was spirochaetosis due to *S. theileri* which is believed to play no part in the aetiology of uitpeuloog as a clinical entity. Up to this stage of the investigations the definite aetiological agent had not been identified.

It is believed that nutritional and environmental factors are at most of minor aetiological importance.

Guinea-pigs and mice were refractory to infection.

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