Studies on Bluetongue

I. Infectivity of the Virus in the Sheep Ked, Melophagus ovinus (L.)

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It is a well-known fact that the virus of bluetongue is transmitted in the field by blood-sucking flies of the genus *Culicoides*. The sheep ked, *Melophagus ovinus* (L.) is another member of the same Order which feeds on blood. It was therefore hypothesized that this disease might also be transmitted by keds. The details and preliminary findings of a series of experiments conducted to test this hypothesis are the subject of this paper.

The condition described has been called "experimental" bluetongue since field cases are unknown in Canada. Clinical symptoms, gross pathology, and histopathology, however, are consistent with those reported from natural cases of bluetongue in areas where the disease occurs. (1) (2).

Materials and Methods

The bluetongue virus (Cyprus strain) used in these experiments was kindly supplied to us by the Director of Veterinary Services, Union of South Africa, in early 1960. It was received as third-passage egg material, and was passaged twice more through eight-day embryonated chicken eggs. Egg inoculation was made onto the chorioallantoic membrane (C.A.) and incubation was at 33.5°C. (3). Penicillin and streptomycin were added to make a final concentration of 500 i.u. and 1 mgm. respectively per 1 ml. of inoculum. At the completion of these passages the virus was producing 100% mortality of embryos in four days, and the embryos were bright red in colour with thickened and haemorrhagic membranes and yolk sac. Chorioallantoic membranes were aseptically harvested and stored at -50°F.

Following the egg passages Sheep I was inoculated subcutaneously (S/C) with 5 ml. of a 10% suspension in buffered saline (pH 7.0-7.1) of ground C/A membranes to which penicillin and streptomycin were added as above. This animal developed a temperature elevation to 104.5°F. on the ninth post-inoculation day and was bled at this time. Clinical symptoms prior to death on the twelfth day following inoculation included conjunctivitis, purulent nasal discharge, stiffness of the neck, oedema of the lips and submaxillary region, hyperemia of the oral mucous membranes, and glossitis. The blood collected from Sheep I was defibrinated and blended with sufficient buffered saline to render it free-flowing, treated with antibiotics, and 5 ml. were inoculated S/C into Sheep II. This sheep developed an elevated temperature (106.0°F.) five days later, and was killed *in extremis* on the 12th post-inoculation day.

Blood drawn from Sheep II at the peak of temperature was inoculated in a similar manner into Sheep III, and its temperature rose to 105.6°F. ten days later. Blood was collected, defibrinated and injected into Sheep IV. This last sheep underwent a thermal response which reached a peak of 105.0°F. on the seventh day. Keds were removed at this time and held at -50°F. for 267 days.

During the course of its illness Sheep IV exhibited symptoms of experimental bluetongue and died on the 25th post-inoculation day. Gross necropsy findings included hyperemia of the oral mucous membranes, oedema of the chin, lips and submaxillary region and a small amount of gelatinous fluid in the subcutaneous tissues of the neck. Histopathological examination of the brain and tongue failed to reveal any significant changes, but that of the neck muscles showed interstitial oedema, mild granulocytic infiltration, and degeneration as indicated by a loss of striation, swelling, hyalinization and fragmentation.

Eggs inoculated on the C/A membrane with a suspension of spleen, heart, and

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lung of Sheep IV treated with antibiotics and incubated at 33.5°C. showed 88% mortality after four days, with haemorrhagic embryos, and thickened, haemorrhagic membranes. This demonstrated that the agent recovered from the organs of Sheep IV was indistinguishable from the original bluetongue virus in its reaction in eggs.

EXPERIMENT A:

Two hundred and sixty-seven days after the keds were removed from Sheep IV they were thawed, ground in a Tenbroeck grinder with 5 ml. of buffered saline, and treated with penicillin and streptomycin as outlined above. The resultant inoculum was injected into Sheep V using 2.5 ml. I/M and 2.5 ml. S/C. By the seventh post-inoculation day this animal had developed a temperature of 105.4°F. Blood was collected, defibrinated and frozen. On the following day (8th post-inoculation) its temperature was 105.8°F. A purulent nasal discharge was present, there was conjunctivitis, and the oral mucous membranes were hyperemic. Keds were removed and frozen at -50°F.

The inoculum used in Sheep V was checked in small laboratory animals, bacterial media, and embryonating eggs for the presence of other infectious agents, with negative results.

EXPERIMENT B:

Keds taken from Sheep V at the time of clinical illness were held for two days at -50°F. then thawed, ground in saline, treated with antibiotics, and inoculated S/C into Sheep VI. A febrile reaction commenced on the 7th post-inoculation day (105.6°F.) and reached a peak (106.6°F.) three days later. Keds and blood were collected at this time and held at -50°F. Symptoms typical of experimental bluetongue were exhibited and this sheep died on the 11th post-inoculation day.

EXPERIMENT C:

Keds taken from Sheep VI at the height of febrile response and frozen for 113 days were prepared and inoculated S/C into Sheep VII. A peak temperature of 106.8°F. was reached on the 7th post-inoculation day. On this day hyperemia of the oral mucosa was evident. In succeeding days oedema of the face and lips, rhinitis, purulent nasal discharge, oedema of the chin and a marked lameness appeared. This animal did not die; symptoms regressed until the sheep was clinically normal on the 15th post-inoculation day.

EXPERIMENT D:

On the twenty-first day after inoculation, Sheep VII, which had by then fully recovered, was challenged with virulent bluetongue virus together with a normal control, Sheep VIII, in order to demonstrate immunity. The challenge material used was defibrinated blood obtained at the peak of febrile reaction from Sheep III (i.e. 2nd sheep passage). This challenge material was administered both I/M and S/C in 5 ml. amounts. Throughout a 21-day observation period Sheep VII remained clinically normal. Sheep VIII, however, showed a temperature rise to a peak of 106.8°F. on the 7th post-inoculation day, followed by hyperemia of the oral mucosa, rhinitis, purulent nasal discharge, glossitis, oedema of the lips and submaxillary region and muscular stiffness. Symptoms regressed and this sheep was clinically normal by the 12th post-inoculation day.

The challenge material was inoculated into 8-day embryonating eggs via the C/A route and incubated at 33.5°C. Mortality of 100% occurred by the 5th day, with the embryos markedly haemorrhagic with thick, oedematous and reddened membranes.

EXPERIMENT E:

Sheep IX was inoculated I/M and S/C with a suspension of ground keds taken from a sheep in the normal supply flock and it remained clinically normal throughout the observation period, indicating that there was nothing inherent in the keds themselves to account for the clinical manifestations observed.

Discussion

This preliminary study was designed to ascertain whether or not keds feeding on infected sheep could maintain the virus of bluetongue within their bodies. The present series of experiments shows that an illness of sheep indistinguishable from bluetongue can be transmitted serially via sheep keds when these are inoculated subcutaneously into susceptible sheep. Results demonstrate that there is no inherent virucidal factor in either their gastric juices or other secretions for this virus. This holds even when the material is frozen for an

extended period of time. The clinical disease produced by ked inoculation is identical to that produced by blood inoculation alone, and elicits an immune response in the host which protects it from challenge with virulent blood.

Further experiments are being conducted to show the importance of keds in the epizootiology of bluetongue disease.

Summary

A series of experiments is reported which shows that the sheep ked *Melophagus ovinus* (L.) can harbour the virus of bluetongue. A series of 3 transmission experiments in which exposed keds were used as inoculum reproduced the disease. This indicates that there is no inherent factor in keds which is lethal to this virus, and suggests a possible role for this ectoparasite in the epizootiology of the disease.

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Oedema in Newborn Pigs

The water-logged condition of the subcutaneous tissues in aborted stillborn and newborn piglets is widely recognized, but there appears to be no published description of the extent and significance of this phenomenon. In this study an analysis is presented of the results of the post-mortem examination of 590 piglets less than 10 days of age. Of these 479 were born alive, and a firm diagnosis was reached in only 145. An examination of 372 newborn piglets for the presence of subcutaneous oedema revealed 114 as being oedematous in the absence of a definite alternative diagnosis. The overall mortality rate among litter-mates of these pigs was 54.5% in cases where very oedematous pigs were found, against 33.2% where specimens were slightly oedematous. A relationship was shown to exist between the presence of subcutaneous oedema and lack of milk in the stomach at autopsy. It is known that there is a low serum protein concentration in apparently normal piglets at birth which is doubled during the first two days of life. The suggestion is made that the low serum protein levels and oedema in newborn piglets is related to the failure of colostrum ingestion. The significance of these findings in relation to data on neonatal hypoproteinemia and possible lines of treatment and prophylaxis are discussed.

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Artificial Pneumoperitoneum for Treating Tenesmus

It has been observed at the Royal Veterinarian College in Copenhagen that following laparotomy of cows suffering from tenesmus the straining has stopped. Although no adequate explanation has been put forth as to why artificial pneumoperitoneum works, nevertheless in 14 cases of retrovaginal tenesmus artificial pneumoperitoneum has been successful. The common therapy in the past for tenesmus has been epidural anaesthesia, however, this is of short duration. To induce artificial pneumoperitoneum air is insufflated using a common trocor cannula mounted on a milk pump with an air filter. Although tenesmus does not always disappear entirely results have been sufficiently successful to recommend this therapy in cases of tenesmus of unknown etiology.

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