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The Transmission of Blue-Tongue and Horse-Sickness by *Culicoides*.

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FOLLOWING upon the earlier investigations into the transmission of horse-sickness (Theiler, 1915), Nieschulz, Bedford and du Toit reported the results of an extensive investigation into the possible transmission of both this disease and a closely allied, but entirely distinct disease of sheep, blue-tongue (Nieschulz, Bedford and du Toit, 1934 and 1937).

This work was based to some extent upon the assumption that, in correlation with such virus diseases as yellow fever and dengue fever, the transmitter or transmitters would be found within the family *Culicidae*. This family includes a number of species which, from the point of view of their biology, fit well with the epizootology of the two diseases. The results of these experiments, however, were entirely negative throughout.

Various views have been expressed from time to time regarding the transmitter. Thus, Williams (1913) was inclined to incriminate *Lyperosia minuta*, observed by him as being very prevalent during an outbreak of horse-sickness in the Sudan. This outbreak was remarkable for the apparent absence of other blood-sucking arthropods. Van Saceghem (1918) has remarked upon the rôle played by such diverse arthropods as ticks (*Rhipicephalus appendiculatus* and *R. evertsi* var. *albigeniculatus*) and members of the order *Diptera*, mentioning certain unnamed species of the genera *Stegomyia*, *Anopheles*, *Lyperosia* and *Stomoxys*. These assertions are made without any supporting experimental evidence and in conclusion it is stated from the observations at Zambi in the Congo some species of *Culicoides* play the principal rôle as transmitter, with *Tabanus pluto* acting presumably as a mechanical agent in disseminating the infection during epizootics. Carpano (1931), in his account of his observations on the disease in Eritrea and Egypt, incriminates the dipterous genera, *Anopheles*, *Aëdes*, *Phlebotomus* and *Simulium* as possible transmitters but favours *Anopheles and Aëdes* (*Stegomyia*) as most probably playing the major rôle.

In this brief review of some of the work done in this field and the theories expressed regarding the transmission, it will be appreciated that at one time or another practically every arthropod which could conceivably play any part has fallen under suspicion.

The present investigation had for its primary object the demonstration of the viruses of the two diseases in question by the emulsification and injections of adult blood-sucking insects caught in the wild state into

susceptible animals. Should positive results be obtained in any one of the families or groups of insects injected this group would then form the subject of closer investigation.

Various methods were used for the collection of insects. These included:

(1) The sweeping of grass and foliage of bushes and shrubs by means of nets.

(2) The collection of biting insects from bait animals at night by means of aspirators, an electric flashlight being used to illuminate insects in the act of feeding.

Both these methods were laborious and not many insects could be captured. Furthermore, no disease was produced by injection of the emulsified insects into susceptible animals.

(3) During the latter part of the 1941-1942 season light traps were brought into operation and with the insects collected by them the first positive results were obtained.

The light trap used at Onderstepoort.

The type of light trap finally adopted is a modification of the New Jersey light trap (see Butts, 1937). It is shown diagrammatically in Fig. 1. The more important modifications are:—

(*a*) The source of light is placed below the catching cage and fan in order that the convection currents induced by the heat of the lamp may assist the draft of the fan. Furthermore, insects are inclined to fly upwards from the low levels of their natural habitat and are more likely to be caught in the vertically ascending column of air and trapped.

(*b*) The extension of the inlet tube or pipe well into the body of the catching cage allows trapped insects to move and remain out of the actual moving column of air, thus greatly reducing mortality. The draft of the fan is not sufficiently strong to hold insects against the fabric of the cage and yet strong enough not to allow of their flying down against it.

(*c*) By placing the cage between the source of attraction and the fan insects are not drawn between the blades of the fan and mortality is further reduced.

(*d*) The dustbin type cover above excludes all rain and the catch is thus well protected.

(*e*) A 75-watt bulb, frosted or clear, was found to give the best results.

(*f*) To ensure the retention of minute insects organdie was used to cover the catching cage. One side of this cage is fitted with a sheet of glass to ensure good visibility.

The operation of the trap is extremely simple. It is suspended at a height of between three and four feet between the lower brim of the conical hood and the ground. This height was found to give the largest catches. The apparatus is connected with the electric mains so that the fan motor and light are in parallel. Sufficient slack in the flex is allowed to permit of the hood being lowered so that the catching cage with its muslin sleeve may be removed or placed in position over the inlet tube. The hood is held in position by means of three spring clips attached to the cylindrical body of

the trap. The trap operates from shortly before sunset until some convenient hour in the morning, when with the fan running, the hood is lowered and the catching cage replaced with an empty one, as only while the fan is running is the catch prevented from escaping.

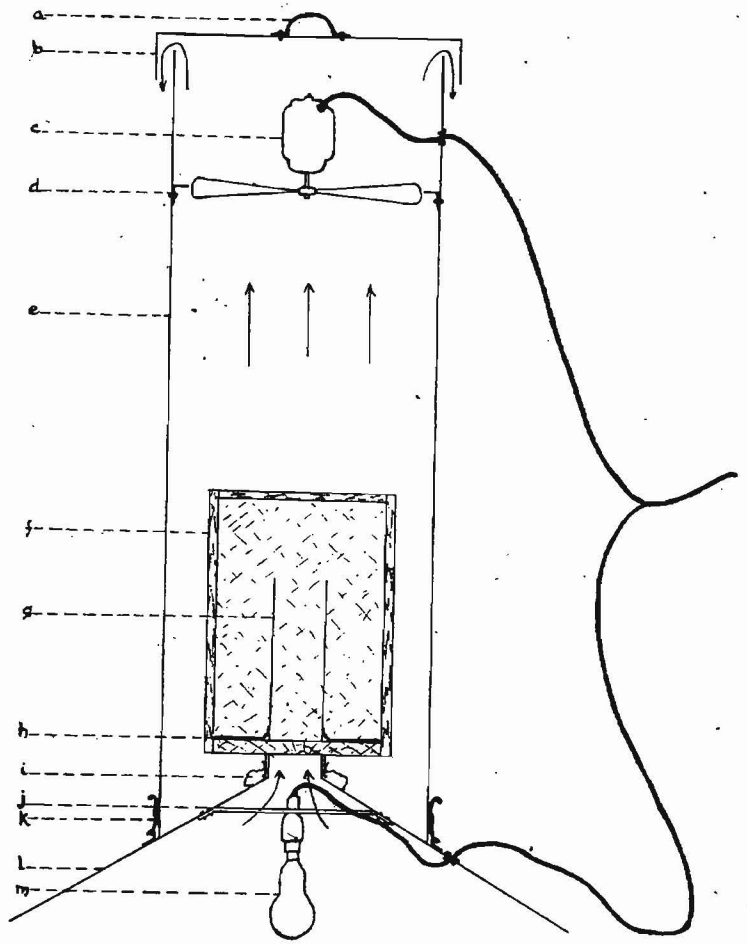

Fig. 1.—Light trap. (*a*) Metal loop for suspending trap. (*b*) Dustbin type cover. (*c*) Electric fan with 7 in. blades. (*d*) Metal flange to allow ¼ in. clearance for fan blades. (*e*) Cylindrical sheet metal body of trap. 8 in. diameter. (*f*) Catching cage. (*g*) Sheet metal inlet pipe or tube. (*h*) Felt or thick woollen cloth glued around opening of catching cage to act as seal between this opening and inlet tube. (*i*) Muslin sleeve. (*j*) Metal bracket to support lamp. (*k*) Spring clip attaching conical hood to body of trap. (*l*) Conical sheet metal hood 17 in. wide below. (*m*) 75 watt electric lamp. Arrows indicate direction of air flow.

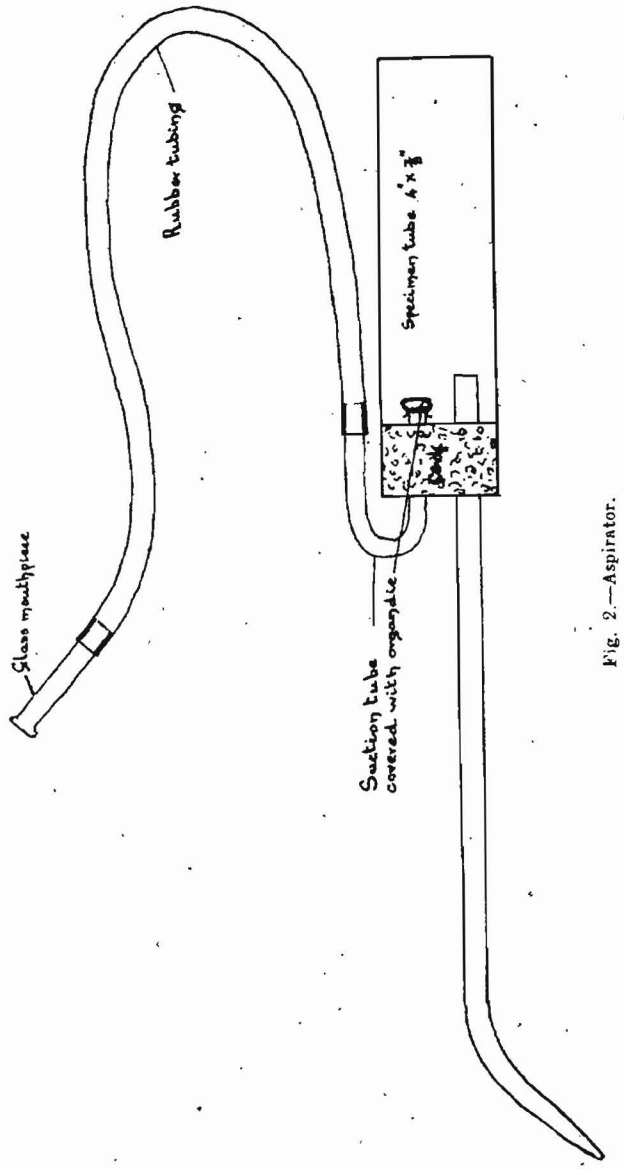

Fig. 2.—Aspirator.

The catch is taken to the laboratory where the cage is placed with the end opposite the sleeve facing a window. The phototropic response of the insects keeps them to the end nearest the source of light where they are easily removed by means of an aspirator, one type of which is illustrated in Fig. 2. In order to facilitate the selection of the *Culicoides* from the variety of insects making up the catch the inlet tube of the aspirator is drawn to a point with an orifice just large enough to permit of the entrance of the largest species of *Culicoides*. In this way mosquitoes, chironomids, moths, muscids and most insects can be excluded. To ensure finally the retention of *Culicoides* only, two methods are used, depending upon whether the insects are intended for injection or feeding experiments. In the former case a small quantity of ether vapour is drawn into the aspirator by inserting the inlet tube into the neck of a bottle of ether and applying gentle suction. The lightly anaesthetised insects are emptied on to a petri dish and rapidly examined under a low power dissecting microscope and the *Homoptera*, *Diptera*, *micro-Lepidoptera*, etc., accidentally drawn into the aspirator, removed. When the *Culicoides* are intended for feeding experiments the contents of the aspirator are liberated into an empty cage where the insects spread themselves sufficiently against the fabric sides to allow of the requisite number of *Culicoides* being selected by means of an aspirator.

Methods of injecting and storing *Culicoides* under Experimental Conditions.

For purposes of injection the *Culicoides* were finely ground by means of a pestle and mortar with the addition of a small quantity of 10 per cent. normal horse or sheep serum in saline and a little sterile quartz sand. After grinding, further serum saline was added and the emulsion spun in a centrifuge at 3,000 r.p.m. for half an hour to remove larger particles of suspended matter. Injections were made intravenously but some animals showed symptoms of shock and several deaths occurred in sheep until a standard procedure was adopted which gave entirely satisfactory results. This consisted of an initial desensitization by injection of 5 c.c. emulsion subcutaneously, followed, half an hour later, by a 10 c.c. intravenous infecting dose.

For storage purposes the *Culicoides* were kept in wooden cages, 5 in. by 5 in. by 5 in., covered with organdie and provided at one side with a glass panel to afford good visibility. The cages were stored in a room kept at 80 per cent. relative humidity and at a temperature of between 78 and 79° F. Food in the form of 10 per cent. sugar solution, soaked raisins and slices of apple was offered and appeared to be readily taken by the insects, and, in additon, water was provided by soaking small balls of cotton wool covered with muslin and suspending these from the roofs of the cages. No final technique has been evolved yet for the storing of the insects and the mortality during storage is still very high, due, principally, to the fact that the insects are inclined to stick to any moist surface.

Methods of feeding *Culicoides* on Experimental Animals.

The method adopted was similar to that described by Nieschulz and du Toit (1934). On account of the minute size of the insects it was necessary to modify the small cages. The mosquito netting was replaced by organdie which was attached to the inside of the wire framework and one side was left open. One end of the cage was provided with a small sleeve. The cages

were held in place upon the experimental animals in the manner described in the paper referred to above, the open side being next to the skin. After the cages were in position upon the animals the *Culicoides* were introduced through the sleeve, allowed to feed overnight, and removed the following morning by means of an aspirator through the sleeve. In the case of sheep it was found necessary to shave the skin to ensure satisfactory feeding.

Experiments with the injection of Wild Caught *Culicoides*.

Injection of emulsified insects caught in the wild state, which included various species of mosquitoes and *Culicoides*, commenced in June, 1942. Details of the actual experimental work will form the subject of a more comprehensive paper later and it is proposed at present to deal only with the positive results obtained.

Experiment 1.

Sheep No. 66242. 3rd March, 1943.—10 c.c. of an emulsion of *Culicoides* collected from three light traps on the mornings of the 25th and 26th and the 2nd and 3rd March were injected intravenously into sheep 66242.

Reaction.—On the 9th March, after an incubation period of 6 days, a febrile reaction commenced which lasted for 7 days and showed a maximum temperature of 106° F. on the morning of the 13th March. Typical clinical symptoms of blue-tongue were observed from the 10th March.

On the 15th April, 2 c.c. of blue-tongue virus (sheep 66289, O.P. virus No. 83, collected 10.2.43) was injected intravenously. No reaction resulted from this injection.

Result.—A febrile reaction with symptoms indistinguishable from those of blue-tongue resulted from the intravenous injection of 10 c.c. of an emulsion of wild-caught *Culicoides*. This sheep failed to react to an injection of blue-tongue virus administered one month later. The sheep was kept under observation until 19.5.43.

Conclusion.—A typical case of blue-tongue had been produced by the injection of wild-caught *Culicoides*.

Experiment 2.

Sheep No. 66277; 14th April, 1943.—10 c.c. of an emulsion of *Culicoides* collected from three light traps on the mornings of the 25th, 26th and 30th March and 13th April, 1943, were injected intravenously into sheep 66277.

Reaction.—On the 20th April, after an incubation period of 6 days, a febrile reaction commenced which persisted for 7 days and showed a maximum temperature of 108·4° F. on the afternoon of the 21st. Clincal symptoms typical for blue-tongue were observed.

On the 5th June, 1943, this sheep received 5 c.c. blood of sheep 66230 (see experiment 3) to which it failed to react. It was kept under observation until 21.7.43.

Conclusion.—A typical case of blue-tongue had been produced by the injection of wild-caught *Culicoides*.

Experiment 3.

Sheep 66230; 26th May, 1943.—10 c.c. of an emulsion of *Culicoides* taken from three light traps on the morning of the 26th May, 1942, were injected intravenously into sheep 66230.

Reaction.—On the 29th May, after an incubation period of 3 days, a febrile reaction commenced which persisted for 12 days. Severe clinical symptoms typical for blue-tongue were observed and the animal became progressively weaker and was killed while *in extremis* on the 9th June, 1943.

On the 5th June, 1943, 10 c.c. blood of this animal was injected intravenously into sheep 66520 which reacted severely and died on the 13th June, from a disease indistinguishable from blue-tongue. On the 5th June, blood of sheep 66230 was also injected into sheep 66277(see Experiment 2), which had reacted to an injection of wild-caught *Culicoides*. This injection failed to produce a reaction.

Result.—A febrile reaction with a fatal termination and indistinguishable from blue-tongue had been produced by the injection of *Culicoides* caught in the wild state. Blood from this sheep, taken during the reaction and injected into a susceptible sheep, produced a reaction similar to blue-tongue which ended fatally. A second injection of blood from this sheep injected into a sheep which had reacted to blue-tongue six-and-a-half weeks previously failed to produce a reaction.

Conclusion.—A typical, fatal case of blue-tongue had been produced by the injection of wild-caught *Culicoides*.

Experiment 4.

Horse No. 223; 10th March, 1943.—10 c.c. of an emulsion of *Culicoides* collected from three light traps on the mornings of the 4th, 5th, 9th, and 10th March, 1943, were injected intravenously into horse No. 223. On the 17th March a further injection of wild-caught *Culicoides*, taken on the 12th, 16th and 17th March, was made into this horse.

Reaction. On the 16th March, 1943, a febrile reaction commenced which persisted for 12 days and the clinical symptoms shown, which lasted for some days after the temperature had subsided, were indistinguishable from those of horse-sickness. The reaction ended in recovery.

On the 24th March, 10 c.c. blood of horse No. 223 was injected intravenously into a susceptible horse, No. 226. This animal showed a febrile reaction which commenced on the 31st March, 1943, 7 days later, and terminated fatally on the 3rd April, 1943, with symptoms indistinguishable from those of horse-sickness of the mixed pulmonary and " dikkop " type. Horse No. 223 was kept under observation until the 1st July, 1943.

Result.—A febrile reaction with clinical symptoms indistinguishable from those of horse-sickness of the " dikkop " type resulted from the injection of wild-caught *Culicoides*. Blood of this horse, taken during the reaction, on injection into a susceptible horse, produced a reaction which terminated fatally with symptoms indistinguishable from those of horse-sickness of the " mixed " type.

Conclusion.—A typical case of horse-sickness of the " dikkop " type was produced by the injection of *Culicoides* caught in the wild state. The fact that the infection could be reproduced in a susceptible horse by injection of

blood, and that this second case terminated fatally with ante- and post-mortem symptoms typical of horse-sickness, places the diagnosis of the initial reaction beyond all doubt.

Experiment with the feeding of infected *Culicoides* upon Susceptible Sheep.

Experiment 5.

Sheep No. 68604; 14th September, 1943.—*Culicoides* species, which had been caught in light traps and had been allowed to feed upon sheep No. 68713 on the night of 3-4th September, 1943, during the course of a reaction indistinguishable from blue-tongue, were placed on sheep No. 68604 on the afternoon of the 14th September, 1943. On the 15th September, three living specimens only could be recovered, two of which had engorged and one had apparently not fed.

(On the 27th August, 1943, sheep No. 68713, upon which the *Culicoides* used in this experiment had had their initial experimental feed, had received an injection of stored blood of sheep No. 66520 taken on the 11th June, 1943. A rather mild but typical reaction had resulted.)

Reaction.—On the 21st September, 1943, after an incubation period of 7 days, a febrile reaction commenced which persisted for 5 days. Symptoms indistinguishable from those of blue-tongue were noted which continued after the temperature had subsided.

The sheep is at present still under observation.

Conclusion.—A typical case of blue-tongue had been produced by the bites of *Culicoides* which had been caught in the wild state and allowed to feed upon a sheep reacting to blue-tongue 10 days previously. The two engorged specimens recovered were identified as *Culicoides pallidipennis*. Although the evidence does not justify an absolute conclusion being arrived at, the indications are that this species can act as the transmitter of blue-tongue in sheep.

Experiments with the feeding of *Culicoides* caught in the Wild State.

For purposes of the discussion of the results obtained, which follows, it is necessary to record that large numbers of *Culicoides* and mosquitoes caught in the wild state were fed upon susceptible animals during March, April and May, 1943. In the case of blue-tongue, 23 such experiments were made and in the case of horse-sickness, 15. Details of these experiments will be recorded in a later paper.

The results were entirely negative.

It must be concluded, therefore, that it had not been possible by means of the light traps used to capture specimens of *Culicoides* during March, April and May, 1943, which were in an infective stage and able by bite to transmit blue-tongue and horse-sickness to susceptible animals.

Discussion of Results.

The positive results obtained by the injection of *Culicoides* caught in the wild state clearly indicate that species of this genus of the family *Chironomidae* harboured the viruses of blue-tongue and horse-sickness during March, April and May, 1943.

It must be admitted that on account of the large numbers dealt with and the minute sizes of these midges it was impossible to exclude engorged specimens from the material injected. It might be argued, therefore, that the reactions produced may have resulted from virus contained in the ingested blood of engorged specimens, which may have fed upon reacting animals shortly before capture. This possibility cannot be excluded entirely but the positive results assume greater significance when viewed in the light of (*a*) the consistently negative results obtained by the parallel series of injection experiments in which the various species of mosquitoes caught in the light traps were injected into susceptible animals over the same period, and (*b*) the fact that the positive results obtained coincided with the particular part of the summer months known from past experience to be the height of the blue-tongue and horse-sickness season, when the greatest number of infected insects could be expected.

The entirely negative results obtained by feeding on susceptible animals *Culicoides* caught in the wild state, some of which could reasonably be expected to have contained virus in view of the positive results obtained by injection, cannot be explained satisfactorily at this stage. The findings of Smith, Halder and Ahmed (1940 and 1941), regarding the retarding effect of successive blood meals upon the development of *Leishmania donovani* in *Phlebotomus argentipes*, may be of application in the present instance but further work is necessary before any comment can be made. In this respect the successful transmission of blue-tongue by the bites of *Culicoides*, which had been stored for 10 days and fed during this period upon sugar solution and fruit juices only with no access to blood, may be of significance.

Summary and Conclusions.

An account is given of an investigation into the transmission of blue-tongue and horse-sickness conducted at Onderstepoort during 1942 and 1943.

Three positive infections with blue-tongue and one with horse-sickness, following the injection of emulsions of wild-caught *Culicoides* into susceptible animals, are recorded.

The successful transmission of blue-tongue by the bites of *Culicoides*, which fed on experimentally sheep 10 days previously, is described.

The identity of the diseases in question had been established clinically, by subinoculations into susceptible animals and by immunity tests with homologous strains of the particular virus where possible. The author is quite confident of the correctness of the diagnoses of the disease conditions produced.

The special light trap used in the investigation for the trapping of *Culicoides* and the technique of handling and storing these insects are briefly described.

It is concluded that certain species of the genus *Culicoides* are capable of becoming infected with and transmitting to susceptible animals by bite the virus of blue-tongue of sheep.

From the evidence advanced it is anticipated that the transmitter or transmitters of horse-sickness will be found within the genus *Culicoides*.

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Some Remarks on Black Quarter Vaccines.

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FOR some years past very little has been published in South Africa on black-quarter vaccines. A number of advertising leaflets have been issued by commercial firms supplying vaccines. Such leaflets are not always reliable. Some experimental evidence on the efficacy of the vaccines which are being used at the present time will, therefore, be of interest.

These vaccines are of two types, the cultural aggressins and the precipitated bacterins.

Cultural aggressins are clear, germ-free filtrates of cultures of *Clostridium chauvoei*. This type of vaccine was issued by the Onderstepoort Laboratory from 1922 to 1934.

Bacterins are vaccines which contain, in addition to the antigenic substances found in the filtrates, the dead blackquarter bacteria. At present such bacterins are treated with alum or aluminium hydroxide. A heavy deposit is thereby produced, which contains the antigenic substances. It is assumed that these are continuously being liberated in the animal body in small quantities over a considerable period of time. In this way the effect of numerous vaccinations with very small doses is obtained, resulting in a much stronger immunity.

REACTIONS PRODUCED BY THE VACCINE.

Cultural aggressins usually cause a very mild reaction, seldom amounting to more than a slight rise of temperature and a moderate lameness. The precipitated bacterins produce a more marked reaction. This is to be expected, when one considers that they contain a large quantity of solid material, with an irritating substance in it, such as alum or aluminium hydroxide. In addition to a rise of temperature and at times a fairly severe lameness, one may notice a swelling at the site of inoculation. It appears shortly after vaccination as a subcutaneous oedema in the form of a flat swelling, and in the course of two to four weeks becomes hard and fibrous and often takes the shape of a nodule which may persist for many months. As a rule, however, such indurations do not seem to worry the animals. The following may serve as an illustration.