Preliminary Bluetongue Transmission with the Sheep Ked Melophagus Ovinus (L.)*

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ABSTRACT

Five experiments indicated that the sheep ked MELOPHAGUS OVINUS (L.), can transmit bluetongue virus (BTV) in sheep. It was not determined whether these were mechanical or biological transmissions, although the results suggested mechanical transmission.

Sheep keds were manually transferred from a BTV-host sheep to 18 susceptible test sheep. Of these, 10 were positive (5 with mild reactions), 6 questionable, and 2 negative for BTV. Three of the mildly reacting sheep and 3 of the questionable sheep had highly intensified reactions on challenge inoculation. Five of the positive sheep were immune on challenge inoculation. Blood from 2 positive reactors was subpassaged into susceptible sheep, which reacted with typical disease signs.

Introduction

The transmission of bluetongue virus (BTV) in sheep has been associated with the genus *Culicoides*, a blood-sucking fly commonly referred to as a gnat or biting midge. However, this insect has not been determined to be the only vector. The sheep ked, *Melophagus ovinus* (L.), which frequently infects sheep, is a common blood-sucking wingless fly belonging to the same order as *Culicoides*. Therefore, it was theorized that the sheep ked might also play an important role in bluetongue (BT) transmission. Preliminary studies on this hypothesis were carried out and are reported here.

Materials and Methods

The BTV used was the California BT-8 isolate serially passaged in sheep. Blood from the second, third, and fourth passages was used and preserved in an equal volume of O.C.G. solution[1].

Susceptible 8- to 18-month-old sheep were obtained from a closed flock. Experiments were conducted in insect-proof isolation rooms. The sheep keds were from BT-susceptible sheep, averaging 50 to 500 keds/sheep (10). These animals had not been previously sprayed with any insecticide.

Hemograms were made on daily blood samples. Clinical signs of disease and hematologic and gross pathologic changes for BT were consistent with those previously reported (5).

Serum neutralization tests in cell cultures were conducted according to a method previously described (3).

Experiments and Results

Experiment 1. — About 60 sheep keds were established on a normal sheep, which was then inoculated subcutaneously with 4 ml. of virus inoculum. Five sheep keds

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[1]An anticoagulant preservative solution consisting of potassium oxalate, 5 Gm.; phenol, 5 Gm.; glycerin, 500 ml.; and distilled water, 500 ml.

were removed daily and transferred to a susceptible sheep from 10 through 35 days after inoculation (DAI). The temperature rose to 104 F. on the eighth day after receiving the initial ked transfer (DAKT). Following 14 days' observatian, a tentative BT diagnosis was made, based on clinical signs of a slight temperature rise at DAKT 8 and mild BT lesions in the oral mucous. The clinical diagnosis of BT was confirmed by a serum neutralization test (2) and the animal's resistance to a challenge inoculation of homologous virus.

Experiment 2. — Two hundred sheep keds were placed on a BT-susceptible splenectomized sheep, which was then inoculated subcutaneously with 10 ml. of blood virus in O.C.G. At DAI 10, 25 keds were transferred to each of 2 sheep, 1 of which had been splenectomized. Additional transfers were made daily on DAI 11 through 14, bringing the total transfer population to 100 keds/sheep. At DAKT 8, 10 ml. of blood was withdrawn from each of the 2 sheep and subpassaged into 2 additional BT-susceptible animals.

The 4 principals all developed mild BT mouth lesions, moderately elevated body temperatures, and slight to marked leukopenia. On the basis of the clinical response, a tentative diagnosis of BT was made.

The 2 sheep receiving sheep keds from the virus host were given challenge inoculation of known BTV. Both reacted with severe signs and lesions of BT. Preceding the challenge inoculation with homologous virus, 230 sheep keds were removed and transferred to a susceptible 7-month-old lamb. A marked but transient leukopenia occurred on DAKT 8. The sheep developed mild BT lesions and a clinical diagnosis of BT was made. The immunity of the sheep was not challenged, and the ked infestation was not removed. Instead, the animal was isolated for the next experiment. A serum neutralization test on the 21st DAKT was negative for BTV antibodies.

Experiment 3. — A donor sheep with a natural infestation of 200 keds received another 300 keds from BT-susceptible sheep. The donor animal was then inoculated with 12 ml. of BTV (blood in O.C.G.), modified by 16 hours' incubation at 37 C. with 0.02% trypsin. At DAI 10, 100 keds were transferred to each of 4 sheep, which had the following history: sheep 1, a BT-susceptible 8-month-old lamb; sheep 2, the lamb held in isolation from experiment 2; sheep 3, 1 of the 2 sheep from experiment 2 that had received a subpassage of blood that possibly contained BTV; and sheep 4, an animal that received 10 ml. of an oral blood virus inoculum 25 days previously

Sheep 1 developed a mild positive reaction. When given challenge inoculation of homologous BTV, it died of BT 24 days later. No clinical reaction was observed in sheep 2; however, when the immunity was challenged with homologous BTV, the animal reacted with typical BT signs and died of BT 8 days later. Sheep 3 was positive for BT transmission, as evidenced by characteristic BT signs and withstood challenge inoculation of a known homologous virus. Subpassage of blood obtained from this animal on DAKT 12 produced BT disease. Sheep 4 developed mild clinical signs of BT but reacted to challenge inoculation with homologous BTV. This sheep had no BTV antibodies on the 21st DAKT but had an SN index of 4 plus following challenge.

Experiment 4. — Three noninfested BT-susceptible ewes (1,2,3) were placed in an isolation room. Keds that had not fed on infected sheep were placed on these ewes. Complete hemograms were made to determine if keds feeding on susceptible sheep would alter the hemograms. No abnormal clinical reactions or variations in the hemograms were observed during a 21-day period. Sheep 1 and 2 were removed to another isolation room and 200 keds were established on sheep 1, which was then inoculated intradermally with 0.2 ml. of blood virus inoculum daily for 10 days. Five sheep keds were transferred to sheep 2 at DAI 6 to 8. Clinical signs of BT were observed in this animal beginning on DAKT 4 and terminating on DAKT 12 when the animal died. Subpassage of its blood, obtained on DAKT 7, produced typical BT disease in 2 susceptible sheep.

Experiment 5. — Nine sheep were used to study the possible optimal circumstances for BT transmission by keds. Of these sheep, 2 were positive and 7 were negative for BT transmission.

An adult sheep ked was allowed to take a blood meal on a BT-susceptible sheep, then starved for 3 days (2), and then allowed to take a blood meal on a BT-infected sheep. The ked was then taped, under gauze, on the ear of a susceptible sheep. A marked leukopenia developed on DAKT 3, and no further clinical response was noted until DAKT 19 when the sheep had clinical signs of BT. The sheep did not develop BT following challenge inoculation with homolo-

gous BTV.

Fifty sheep keds were allowed to feed on a BT-infected sheep until the onset of leukopenia in the animal. Twenty-five blood-fed keds were transferred to a BT-susceptible sheep for 2 consecutive days. Typical BT signs developed on DAKT 8, and the animal withstood challenge inoculation. Forty sheep keds were removed from this animal on DAKT 8 and transferred to another BT-susceptible sheep, which developed mild clinical signs of BT. However, the animal did not withstand challenge inoculation and an SN test on the 21st DAKT was negative but 21 days after challenge the sheep had an SN index of 4 plus.

Discussion

The experiments indicate that the sheep ked is capable of BT transmission; however, the data are more suggestive of a mechanical rather than a biological transmission. Sheep keds feed regularly only about every 36 hours on sheep in the field and also when maintained in the laboratory at 30 C. (6). On this basis, biological transmission remains a possibility.

A variety of experimental conditions were employed in our experiments in order to utilize the information reported by Alexander, *et al* (1). They reported on their routine diagnostic procedure for bluetongue where a suspect blood BTV sample was negative only after the third serial passage in sheep was negative. It was their usual experience to find that subinoculation of blood from a BT sick sheep produced merely a mild febrile reaction in a susceptible sheep. However, further subpassages produced the usual severe clinical syndrome. We have observed similar findings in our BT diagnostic work. In these instances the suspect blood virus sample was inoculated into a BT-susceptible sheep and caused the development of questionable or mild clinical signs of the disease. Usually 3 serial subpassages of blood obtained at the height of the clinical response was necessary to establish good virulence and immunity. A challenge of the immunity in these sheep with known BTV often resulted in intensified clinical signs and death in the first and sometimes the second sheep inoculated.

Three of 6 sheep that were questionable and 3 of 5 that developed mild clinical BT responses had an exaggerated clinical response on challenge inoculation. The clinical response was much more severe than that seen in typical experimental BT disease. (5) The 5 sheep with mild clinical BT responses all were negative for BT antibodies on DAKT 21 but they all had an SN index of 4 plus following challenge with the known homologous BTV. This may suggest previous virus contact since unpublished data on 50 BTV inoculated sheep at the Denver laboratory gave SN indexes of 1.0 to 4.7 with a mean of 3.0.

A greater percentage of positive BT transmissions might have occurred if all experiments had been done under the phase of susceptibility to the ked. According to previous reports, (7, 8, 9) every sheep has a phase of susceptibility (ked increase) and a phase of resistance (ked decline). Ked numbers usually increase to peak levels in January and February, decline steadily until June, and remain at low levels until September.

Other workers (4) found the sheep ked capable of harboring BT virus and reported that no detrimental BT viral factor was present in the ked. Therefore, further work is needed to find the importance of sheep keds in the epizootiology of BT disease.

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