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Isolation of the Bluetongue Virus from Texas Sheep—Culicoides Shown to Be a Vector

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IN A PREVIOUS paper,[1] two epizoötics (occurring in 1948 and 1951) of the disease then called "soremuzzle" were described and the possibility of its being bluetongue was suggested. Later, a similar condition was reported in California and specimens sent from that state to the Onderstepoort laboratory in the Union of South Africa resulted in a positive diagnosis of bluetongue.[2]

During 1952, this disease was diagnosed on only four ranches in Texas. Then, in mid-May of 1953, we investigated what proved to be the beginning of another epizoötic. Within the following four-month period, it had extended to many points within the area shown in figure 1.

Since the major portion of the Texas sheep population is restricted to those regions in West Texas known as the Edwards Plateau and the Trans Pecos, it naturally follows that an epizoötic involving only sheep would be restricted to this area; but it is of particular interest that the three epizoötics had their origins along the Rio Grande River, which constitutes at once the southwestern boundary of the area and the international border. Here, the parallel of latitude is approximately 29 degrees, and much warmer climatic conditions prevail in early summer than at 32 degrees 30 minutes latitude, the northern extent of the area. Since spread of the disease is believed to depend entirely upon an insect vector which is inactive during the winter months, it is not strange that the outbreaks have begun at the southern extent of the area. All three epizoötics began in May or June and terminated during one of the autumn months.

It should be understood that in each year the distribution of bluetongue was undoubtedly greater than that shown in figure 1. We have recorded only the first-hand information which was available to us through field trips, clinical examinations, and personal communications with veterinary practitioners, county agricultural agents, and ranchmen. Hearsay reports would have extended the affected area to many more West Texas counties; and, in at least one year, the area would have extended well into a neighboring state.

ISOLATION AND PROPAGATION OF ONE STRAIN OF THE VIRUS IN TEXAS

During the years 1950 through 1952, blood samples from affected sheep were collected and stored in the oxalate-phenol-glycerine solution reported by Neitz[3] to have kept bluetongue virus viable for more than twenty-five years at room temperature. During subsequent attempts to isolate the agent from this preserved blood by means of animal inoculation, the scarcity of susceptible sheep caused many of the blood specimens to be pooled, and it was from one such pooled specimen that a mild form of clinical bluetongue was transmitted to

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The antibiotics for virus procedures were furnished by Merck & Co., Inc., Rahway, N. J.

susceptible sheep. Neitz and Riemerschmid[4] have demonstrated the intensifying effect of sunlight on the course of this disease and the severity in this case was no doubt influenced by the fact that the sheep were stabled during the entire period. Stabling could not be avoided because the work was done during the summer months and it was therefore necessary to guard against a field outbreak. After a further passage in sheep, the infective agent was passed to embryonating hen eggs where it adapted readily under the conditions to be described.

Fertile hen eggs incubated for six to eight days at 38 C. were inoculated via the yolk sac route with a 10^{-1} dilution of citrated infective blood in infusion broth, with 0.5 ml. of inoculum being used for each egg. To this and all inoculums mentioned hereafter, streptomycin, 12 mg. per milliliter, was added and an incubation period of at least ten minutes was provided prior to inoculation of the eggs.

Since Alexander[5] has shown that the bluetongue virus has an optimum growth temperature below that which is normal for hen eggs, inoculated eggs were incubated in duplicate in the only two available incubators, both of forced-draft type. One operated at 38 C.; the other was set for 33.6 C., but since the work was being done during the summer months, afternoon temperatures in this incubator were known to reach as high as 36.7 C. The agent failed to propagate at 38.0 C., so subsequent work was conducted at the lower, but variable, temperatures, to which the agent became adapted within the first few generations.

TABLE 1—Protocol to Show Propagation of Sonora Strain of Bluetongue Virus by Serial Passage Through Eggs

Egg generation	Results following inoculation						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	0/14	0/14	0/14	2/14	4/14	10/14	10/14
2	0/11	2/11	8/11	11/11	.....	.....	.....
3	0/12	0/12	0/12	11/12	12/12	.....	.....
4	1/12	2/12	9/12	10/12	10/12	10/12	10/12
5	0/6	2/6	4/6	4/6	4/6	5/6	5/6
6	0/6	0/6	0/6	1/6	1/6	2/6	3/6
7	0/6	0/6	5/6	5/6	6/6	.....	.....
8	0/6	1/6	4/6	5/6	5/6	5/6	5/6
9	0/6	1/6	5/6	5/6	6/6	.....	.....
10	0/8	6/8	8/8	.....	.....	.....	.....
15	0/11	0/11	8/11	10/11	11/11	.....	.....
20	0/5	2/5	5/5	.....	.....	.....	.....
25	0/11	4/11	7/11	11/11	.....	.....	.....
30	0/5	2/5	4/5	4/5	5/5	.....	.....
35	0/6	5/6	6/6	.....	.....	.....	.....

Note — 10/12, 8/11, etc., means 10 dead, 12 inoculated; 8 dead, 11 inoculated, etc.

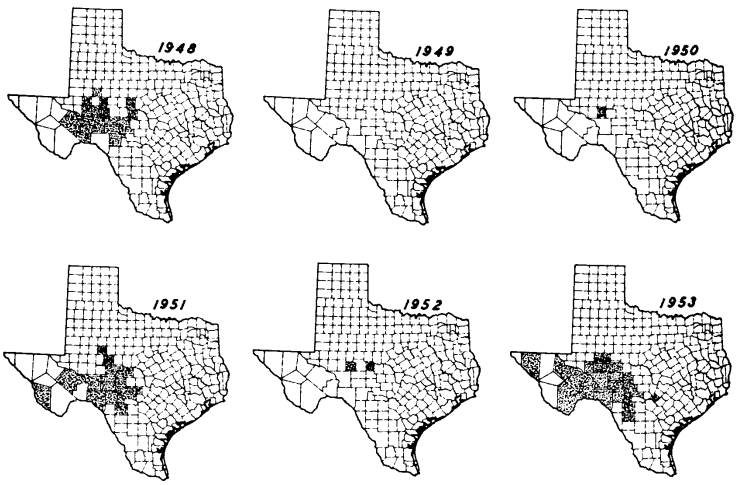


Fig. 1—The incidence of bluetongue in Texas is shown by shaded counties.

The advent of autumn made possible a uniform incubation temperature of 33.6 C. for serial egg passages beyond the twenty-fifth. Routine egg passages were made via the yolk sac route with pooled two- to four-day* dead embryos ground in a Waring blendor and diluted 10^{-1} with broth, 0.5 ml. of inoculum being used per egg.

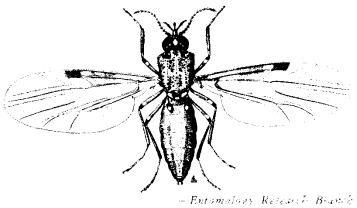
Intravenous inoculation of susceptible lambs with tenth-passage embryo emulsion elicited on the seventh to ninth days a thermal reaction only (maximum temperature, 106.4 F.). Challenge three weeks later with homologous virus demonstrated immunity. Inoculation of lambs with twenty-first-passage embryo emulsion resulted in a questionable hyperthermia only, with a maximum temperature of 104.9 F. on the sixth and eighth days. Immunity was again demonstrated by challenge with the homologous virus. Susceptible lambs which were inoculated with fortieth-passage embryo emulsion exhibited even less reaction, but developed an immunity that withstood challenge by the homologous virus. (Both of 2 controls inoculated with the challenge material developed pronounced clinical bluetongue and 1 died.) Stabled sheep were used for the foregoing passages. Tentatively, the strain just described is called the "Sonora" strain.

POSSIBILITY OF MULTIPLE STRAINS OR TYPES

When Dr. R. A. Alexander, director of veterinary services for the Union of South Africa, visited this state early in 1953, he examined specimens which had been preserved from the 1951 epizoötic and stated the opinion that the three zones of coronitis plainly visible on one of the specimens constituted evidence of the existence of as many strains of bluetongue in Texas. Subsequently, it has been our observation that multiple zones are not uncommon, and occasionally a ranchman will relate that his flock had seemed to recover only to be affected again a week or so later. New strains are being sought within the limitations imposed by the availability of susceptible sheep, equipment, and personnel.

The infective agent was recently isolated from a sheep artificially infected by means of the vector described below and from two field outbreaks of bluetongue approximately 140 miles from our laboratory and 50 miles from one another. At the time of this writing, each has been passaged through several egg generations with considerable difficulty, but since their relationship to the Sonora strain or to one another has not been determined, the matter of their identification and propagation will be reserved for a future report.

*Embryos which died two to four days after inoculation.



—Entomology Research Branch

Fig. 2—A specimen of Culicoides variipennis (Coquillett).

SEARCH FOR A VECTOR

It was concluded by Du Toit[6] that species of the sylvanic Culicoides midge are transmitters of bluetongue in South Africa, and no other vector has since been reported.

Acting on this information, the chief of the Animal Disease and Parasite Research Branch requested that the Entomology Research Branch make a taxonomic survey and biological study of this genus in the Southwest. The work is now under way, and the senior author, coöperating in the survey by operating a New Jersey-type mosquito trap near the Sonora laboratory, has used the trap independently to make catches of Culicoides for transmission trials in sheep.

A description of negative trials would serve no purpose, but on one occasion subcutaneous and then intravenous injections into stabled sheep of a broth emulsion of *Culicoides variipennis* females resulted in the production of clinical bluetongue on the seventh day. These specimens were taken from the trap early in the morning, identified and selected under the dissecting microscope, ground finely with ground glass in a mortar, sterile broth added, centrifuged at 3,000 r.p.m. for ten minutes, and the supernatant fluid injected fifteen minutes after the addition of 12 mg. of streptomycin and 10,000 units of penicillin per milliliter of fluid. The inoculum was proved to be bacteriologically sterile.

On another occasion, a similar transmis-

sion trial resulted in a febrile reaction from the seventh to twelfth day (maximum temperature, 105.4 F.), with buccal hyperemia the only lesion noted.

The species of Culicoides used in both instances was by far the predominating one at this location at the time the trials were conducted. From a catch made in the same light trap on an intervening night, Wirth[7] identified a total of 200 *C. variipennis* and only 1 of another species, *Culicoides multipunctatus*. These two instances of transmission are not claimed to be entirely conclusive but are deemed significant in view of the South African work.

SUMMARY

Epizoötics of bluetongue in Texas sheep are reported to have occurred during the summers of 1948, 1951, and 1953.

The isolation and propagation of one Texas strain is briefly described. A lower-than-normal incubation temperature has been used for serial egg passages and the agent apparently has become attenuated.

Transmission of clinical bluetongue in sheep was accomplished by the injection of an emulsion of *Culicoides variipennis*.

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Recent Epizootics

Foot-and-mouth disease (aftosa) was reported on February 26 *in carabao on Luzon, P. I.;* the type of virus has not been determined. Upon request, the U.S.D.A. assigned Dr. C. U. Duckworth to assist in its control.

Aftosa control in Mexico is proceeding satisfactorily; no new cases have appeared in 1954.

Scrapie in New York (Erie County) was reported on February 15 by the U. S. D. A. This virus neurosis of sheep was eradicated in California and Illinois a year ago but has recently spread to Ohio where control by quarantine is being attempted.

Vesicular exanthema in Arkansas was verified February 12, when authorities were called by an owner who had recognized the symptoms from a telecast of a U.S.D.A. film. Before then, V. E. was confined to California and seven northeastern states. Arkansas is one of the four infected states where cooking of garbage is not required.

Hypodermic Injection Centennial

Dr. Alexander Wood, Edinburgh, was the first to give, in 1853, a subcutaneous injection in the modern sense (*Brit. M. J.,* Nov. 28, 1953). Twenty drops of a solution of muriate of morphia were given to an elderly lady suffering with neuralgia, who could not take opium by mouth. Intravenous injections, however, had been given through a quill since 1657.

1953 Proceedings Book Correction

In the article, "Etiology of Chronic Respiratory Disease" by H. Van Roekel and Olga M. Olesiuk, Department of Veterinary Science, Massachusetts Agricultural College, Amherst, which was presented before the Section on Poultry at the Toronto convention, the usual abbreviation "CRD" for the disease appeared in lower case "crd" (*see* pages 289-301, 1953 Proceedings Book).

It should be noted that Dr. Van Roekel checked the galley proofs of the article and indicated "crd" should be capitalized in each instance. However, as a measure of economy, the editorial department decided that the expense of resetting about 100 lines of the article was hardly justifiable and so the incorrect "crd" abbreviations were allowed to stand.

This correction notice is printed in fairness to the authors, whose original and correct manuscript was edited for the printer by an inexperienced person. The editorial department greatly regrets the circumstances.

The History of Pseudorabies in the United States

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THE HISTORY of a disease before its acceptance as an entity is, with few exceptions, beyond reach of the scientific approach. The exceptions arise from the recognition of signs or ecology of disease which are sufficiently unique to permit tentative diagnosis even from reports written several hundred years earlier. The stories which have been pieced together on rabies and syphilis suggest the fruitfulness of this method of "historical postmortem" to those interested in the evolution of disease.

Accounts of what was probably pseudorabies during the century prior to its recognition are the topic of this paper. Pseudorabies of cattle is a rapidly fatal disease characterized by pruritus. The clinical picture is usually sufficiently unique for diagnosis based on clinical observation alone.

Aujeszky,[2] a Hungarian physician and veterinarian living in Budapest, is credited with the first description of pseudorabies which he published in 1902. He demonstrated that a disease occurring naturally in cattle, dogs, and cats was transmissible to rabbits in which it produced characteristic signs which terminated in death. From that year until 1930, some 28 papers on pseudorabies appeared. Aujeszky's disease, infectious bulbar paralysis, or mad itch as the entity was variously called, was reported from numerous places in Europe and Brazil, and its presence was suspected in the United States.

Shope,[14] in 1931, established that mad itch occurring in cattle in Iowa was the same as Aujeszky's disease. In a series of papers[15-17] published in the next few years, he showed that a mild and usually unrecognized disease of swine was produced by the agent of mad itch or pseudorabies. The virus was infectious for swine and passed readily through the herd. If cattle were pastured with infected swine, the transmission to cattle occurred through an abrasion in the skin but the virus was not transmitted from cow to cow. During the period 1931 to 1940, interest in pseudorabies increased considerably—199 papers appeared—but in the next decade, 1941 to 1950, investigators apparently turned to other problems and only 60 papers on the subject were published.

In the United States, pseudorabies is known to exist in a large section of the Middlewest. The virus, which has been isolated in Iowa, Illinois, Wisconsin, Missouri, and Minnesota, is probably more common than the incidence of the overt disease would suggest. An antibody survey[17] made in 1934 revealed that 21 of 23 pools of serum obtained from midwestern establishments producing hog cholera antiserum contained pseudorabies neutralizing antibodies. Failure to recognize pseudorabies in cattle in other sections of the country is not evidence of its absence. The virus was isolated recently in four of the southeastern states—Louisiana, Alabama, Georgia, and Florida.[3]

As Shope stated in 1931, pseudorabies had been present in the United States for an unknown length of time. Usually known as mad itch, it was considered by many to be a form of hemorrhagic septicemia. White,[18] author of a textbook of veterinary medicine published in 1917, considered mad itch synonymous with pseudorabies, but without experimental evidence. The Alabama outbreak, which he cites, an instance of pruritus of horses and mules terminating rapidly in death, if pseudorabies was certainly not typical of the mad itch of earlier or later observers which usually occurred in cattle. A similar account of a fatal disease of mules characterized by pruritus was reported in 1914 by the Florida Board of Health.

Of seven books dealing with livestock disease which were published in America between 1840 and 1900, two refer to mad itch. McIntosh[19] described it as "dearrangement of cattle present more some years than others" and said to be caused by eating cornstalks. Cole[4] in 1847 gave the symptoms as licking and rubbing, with death in ten to twelve hours.

The farm journals of that era have more information. English[5] reported to the *American Agriculturist* that mad itch, although uncommon in the neighborhood, had destroyed 9 head of cattle in Auglaize County, Ohio, during the last two weeks of September in 1857. He referred to the general belief that it was caused by allowing cattle to eat chewed wads that hogs leave after feeding on green cornstalks but asked, if this were true, why the disease did not kill his neighbor's cattle or any the previous year, since running them with hogs was a universal practice. His description was typical: abrasion of the skin by rubbing that increased in violence as the disease progressed and which was associated with general hyperirritability, twitching, bellowing, finally abrasion of the flesh, exhaustion, and death. The latter usually occurred less than thirty-six hours after the first signs. Hartzell[9] of Hancock County, Illinois, another reader of the *American Agriculturist,* also took exception to the swine hypothesis. He claimed that the disease could not be carried by feeding cattle with hogs because many of the beef cattle that had been attacked in his area never ran with

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