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in 1 injection
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Isolation of the Bluetongue Virus from Texas Sheep—
Culicoides Shown to Be a Vector

D. A. PRICE, D.V.M., and W. T. HARDY, D.V.M.

Sonora, Texas

IN A PREVIOUS paper,1 two epizootics (oc-
curring in 1948 and 1951) of the disease
termed "bluetongue" 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 Underwood
laboratory in the Union of South Africa
resulted in a positive diagnosis of bluet-
tongue.

During 1952, this disease was diagnosed
only four times in Texas. Then, in
mid-May of 1953, we investigated what
proved to be the beginning of another epiz-
odec. 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 re-
gions in West Texas known as the Edwards
Plateau and the Trans Pecos, it naturally
follows that an epizootic involving only
sheep would be restricted to this area; but
it is of particular interest that the three
epizootics 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 be-
lieved 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 epizootics 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 undoubt-
edly 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 veteri-
ary practitioners, county agricultural
agents, and ranchmen. Hearsee reports
would have extended the affected area to
many more West Texas counties; and, in
at least one year, the area would have ex-
tended 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-pheno-
glycol solution reported by Neitz.2 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 sus-
sceptible 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

1 From the Texas Agricultural Experiment Station, sub-
vention No. 14, San Antonio, Texas.
2 Neitz, A. D., Eppinger, E. L., and Otis, W. L., "A
Stable Repository Medium, Industrial Research Branch,
U.S. Department of Agriculture, Washington, D.C.
3 Neitz, A. D., "The Isolation of Arbovirus, Anostrangesh
Research Society, Entomology Research Branch, U.S.
Department of Agriculture, Washington, D.C.
4 Histochemical techniques for virus preparation were furnished by
Bax & Co., Inc., Newark, N. J.
susceptible sheep, Neitz and Riemerschmidt 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 all, this work in passage sheep, the infective agent was passed to embryos. Incubation at 38°C (100°F) of eggs was provided prior to inoculation of the eggs.

Since Alexander 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 op-erated 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 38.7°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 Sommer Strain of Bluetongue Virus by Serial Passage Through Eggs

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Note: 10.24% = 1 ml., 20.48% = 2 ml., 5.12% = 5 ml., 2.56% = 2.5 ml., 1.28% = 1.28 ml., 0.64% = 0.64 ml., 0.32% = 0.32 ml., 0.16% = 0.16 ml., 0.08% = 0.08 ml., 0.04% = 0.04 ml., 0.02% = 0.02 ml., 0.01% = 0.01 ml.

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-old embryos (1-2 ml) in a Waring blender and diluted to 10 x with broth, 0.01 ml. of inoculum being used per egg.

Intravenous inoculation of susceptible lambs with tenth-passage embryo emulsion started on the seventh to ninth days after 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 twenty-fourth-passage embryo emulsion exhibited even less reaction, but developed as immunity that withstood challenge by the homologous virus. Both of 2 controls inoculated with the challenge material developed pronounced clinical bluetongue and died.) Stabled sheep were used for the foregoing passages. Finally, the strain just described is called the "Sommer" strain.

Possibility of Multiple Strains or Types

When Dr. R. A. Alexander, director of the Veterinary Services for the Union of South Africa, visited this state early in 1953, he examined specimens which had been preserved from the 1951 epidemic and stated the opinion that the three zones of cornitis plainly visible on one of the specimens contained evidences of the existence of as many strains of bluetongue in Texas. Subsequently, it has been our observation that multiple strains 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 10 miles from our laboratory and 50 miles from one another. At the time of this writing, each has been passed through several egg generations with considerable difficulty, but since their relationship to the "Sommer" strain or to one another has not been determined, the matter of their identification and propagation will be reserved for a future report.

Search for a Vector

It was concluded by Du Toit that species of the syrphid Callidodes mudgei 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, cooperating in the survey by operating a New Jersey-type mosquito trap near the Sommer laboratory, has used the trap independently to make catches of Callidodes 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 Callidodes species 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 5,000 g 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 transmit-
tion trial resulted in a febrile reaction from the seventh to twelfth day (maximum temperature, 106.4° F.), with bacilar hemoptysis the only symptom.

The species of Culexoides used in both instances was by far the predominating species at this location; the other species were not identified. From a catch made in the same light trap on an intervening night, Wirth identified a total of 280 C. restuans and only 1 of another species, Culexoides nitidicornis. These two instances of transmission are not claimed to be entirely conclusive but are deemed significant in view of the South African work.

SUMMARY

Epizootics 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 infection of an unvaccinated Culexoides v. nitidicornis.

References


Recent Epizootics

Foot-and-mouth disease (vaccinia) was reported on February 26 in a ewe on Long Island, L. I.; the type of virus has not been determined. Upon request, the U.S.D.A. designated Dr. C. C. Dukeuck to assist in its control.

African cattle in Mexico is proceeding satisfactorily: no new cases have appeared in 1954.

Syringe in New York (Erie County) was reported on February 16 by the E. S. D. A. This virus strain of sheep was eradicated in California and Illinois a year ago but has recently spread to Ohio where control by quarantine is being attempted.

Foot-and-mouth outbreaks in Arkansas were 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.

Hydropyogenic 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, 1853). Twenty drops of a solution of moriain of morphea were given to a kindly lady suffering with neurasthenia, who could not take opium by mouth. Intravenous injections, however, had been given through a quill since 1657.

1953 Proceedings Book Correction

In the articles, "Histology of Chronic Respiratory Disease" by H. Van Roekel and Olof M. Olafson, Department of Veterinary Science, Massachusetts Agricultural College, Amherst, which were presented before the Section on Pulmonary at the Tetracon convention, the usual abbreviation "C.R.O." for the disease appeared in lower case C.R.O. (see pgs 280-281, 1953 Proceedings Book).

It should be noted that Dr. Van Roekel checked the galleys proofs of the article and indicated "c.r.o." should be capitalized in each instance. However, it may be a measure of economy, the editorial department decided that the expense of relettering about 100 lines of the article was hardly justifiable and in the incorrect "c.r.o." 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

ROBERT F. HANSON, P.D.D.

Medicine, Wisconsin

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 classification, often even from reports written several hundred years earlier. The galleys which have been pieced together on apes and aphantiles suggest the fruitfulness of this method of "historical postmortem" to those interested in the evolution of diseases.

Accounts of what was probably pseudorabies during the century prior to its recognition are the epitome of this paper. Pseudorabies of cattle is an epizootic disease characterized by polyneuritis. The clinical picture is usually sufficiently unique for diagnosis based on clinical observation alone.

Aujeszky's, a Hungarian physician and veterinarian living in Budapest, is credited with the first description of pseudorabies, which he published in 1892. He demonstrated that a disease occurring commonly in cattle, dogs, and cats was transmissible to rabbits in which it produced characteristic symptoms which terminated in death. From that time until 1931, some 26 papers on pseudorabies appeared. Aujeszky's disease, infectious bursal paralysis, or mad itch as the entity was variously called, was reported from numerous flocks in France and Brazil, and its presence was reported in the United States.

Sheep, in 1934, established that mad itch occurring in cattle in Iowa was the same as Aujeszky's disease. In a series of papers published in the next few years, he showed that a hitherto unsuspected disease of swine was produced by the agent of mad itch or pseudorabies. The virus was infectious for guinea pig and varied readily through the herd. If cattle were infected with infected swine, the transmission to little occurred through an abrasion in the skin but the virus was not transmitted from cow to cow. During the period 1935 to 1940, interest in pseudorabies increased considerably—199 papers appeared in the next decade, 1941 to 1950, interest was apparently similar to other problems and 66 papers on the subject were published. In the United States, pseudorabies is known to be a large section of the Middlewest. The virus, which has been isolated in Iowa, Illinois, Wisconsin, and Minnesota, is probably more common than the incidence of the over 100 disease would suggest. An account of a "strange fever" in 1934 revealed that 21 of 23 pools of sera obtained from midwestern establishments producing hog cholera antigen 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.

As Shope stated in 1931, pseudorabies had been present in the United States for an unknown length of time: as early as known as mad itch, it was considered by many to be a form of hemorrhagic encephalitis, white." 1. author of a text book 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 males terminating rapidly in death, if pseudorabies was certainly not typical of the mad itch of earlier or later outbreaks which usually occurred in cattle.

A similar account of a fatal disease of swine characteristic of pruritus was reported in 1934 by the Texas Board of Health.

Of seven books dealing with livestock diseases which were published in America between 1930 and 1950, two refer to mad itch. McKendry described it as "clearances of cattle present more soreness than others" and said it was caused by eating corns. Cole in 1934 gave the symptoms as itching and rubbing, with death in ten to twelve hours.

The term journals of that era have more information. English reported to the American Agriculturalist that mad itch, although unconfirmed in the neighborhood, occurred in a herd of cattle in Arundel County, Ohio, during the last two weeks of September in 1897. He notified the local belief that it was caused by allowing cattle to eat chewed sods that cows have after feeding on raw corns but there was no suggestion that the disease did not kill his neighbors' cattle on the previous year, since running then with bugs was a universal practice. His description was typical: abrasion of the skin by rubbing that it caused in violence as the disease progressed and which was associated with general hyperemia, pruritus, bellowing, finally abrasion of the flesh, emaciation, and death. The latter usually occurred less than three-six hours after the first sign. Harrell of Hancock County, Illinois, another member of the American Agriculturalist, also took exception to the swine hypothesis. He claimed that the disease could not be carried by leading cattle with hogs because many of the beef cattle which had been attacked in his area never ran with
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