BLUETONGUE[1]

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The recognition of a hitherto exotic disease known as bluetongue in the sheep population of a number of our western states (1, 2, 3, 4) has recently aroused the interest of veterinary medicine in the United States. The purpose of this review is to summarize our present knowledge concerning this disease, known previously only in rather remote parts of the world.

Bluetongue is an infectious, inoculable, viral disease of sheep. Cattle and goats may harbor the virus, but they generally fail to show pronounced clinical symptoms. The disease in sheep is characterized by catarrhal inflammation of the mucous membranes of the mouth, nose and intestines, and often by inflammation of the coronary bands and sensitive laminae of the hoofs. There is an excoriation of the epithelium and, later, necrosis of the buccal mucosa; the swollen and inflamed tongue and mouth take on the dirty blue color from which the disease derives its name. Bluetongue is not contracted by contact but is transmitted by biting insects. The etiological agent is a resistant virus which remains viable for many years whether kept in the fluid state or frozen and dried under vacuum. Over 15 virus strains have been isolated, which apparently fall, however, within four or possibly five immunologically distinct groups. Animals carry the virus for weeks or months after recovery and develop immunity to subsequent infection with the homologous strain.

HISTORY

The literature shows that South African investigators have done most of the recorded work on bluetongue. In that country, the disease has seriously menaced the livestock industry for quite some time; probably since the very beginning of sheep farming. Henning notes (5) that the Report of the Cattle and Sheep Diseases Commission for 1876 (6) states: "For many years, if not from the time of the introduction of the Merino sheep into the Colony, there has been prevalent amongst the flocks a disease known as *fever*. This disease is most prevalent during the summer months, and is very much worse in wet seasons." The same report also mentions that fat sheep are more susceptible to *fever*, and that the disease is more often found in valleys and low-lying areas than in high-lying veld. Sheep stabled in sheds during summer nights are described as escaping infection. The morbidity of *fever* was estimated at over 30 per cent, and the mortality of those animals affected at more than 90 per cent. The chief symptoms listed are soreness of the mouth and feet, and the illness is likened to foot-and-mouth disease. In 1933, bluetongue was recognized for the first time as a disease of cattle (7). In these animals it apparently gave rise to a stomatitic infection similar to foot-and-mouth disease.

Hutcheon reported bluetongue in 1881, referring to it as "fever or epizootic catarrh" (8) in sheep; Spreull (9) was the first to study the disease in detail. He proposed a method of immunization by the simultaneous inoculation of immune serum and virulent whole blood. Theiler (10), who was unable to confirm Spreull's work, introduced another method of immunization. He used no antiserum, but only infected blood in which he believed the virus had been attenuated for sheep by several serial passages in the same species. Vaccine prepared by Theiler's formula[2] was employed for nearly 40 years. During this time more than 50 million doses were distributed for general field use by the government laboratories at Onderstepoort (11). In 1946, a chicken embryo propagated, living virus vaccine, quadrivalent in type, developed by Alexander and his co-workers (12, 13, 14, 15, 16) replaced Theiler's vaccine and is still being used.

HOST RANGE

Bluetongue is primarily a disease of sheep. All breeds are susceptible: Afrikander and Persians less so than Merinos; and some British breeds,

[1] Synonyms: Catarrhal Fever, Malarial Catarrhal Fever, Bloutong, Blauw Tong, Besiekte, Vuel Bek.

[2] Potassium oxalate, 5 g; phenol, 5 g; glycerine, 500 ml; distilled water, 500 ml. 1:1 with infected whole blood. Formula from (16).

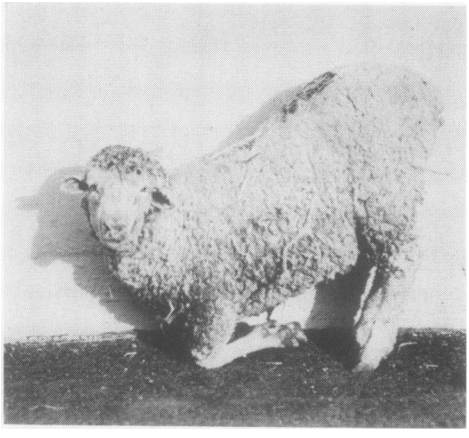

Figure 1. Sheep showing typical signs of bluetongue. All figures are through the courtesy of Dr. Blaine McGowan, School of Veterinary Medicine, University of California, Davis, California.

such as Dorset Horns, more so (17). Young sheep about a year old are most vulnerable and generally develop a severe form of the disease. Sheep born and raised in an enzootic area resist the disease more than those brought in from other regions. As would be expected, the susceptibility of individual animals of the same breed differs markedly. Thus, the pathogenicity of a particular strain of virus cannot be judged by the reaction produced in a single sheep. As a rule, however, virus strains may be classed as virulent or avirulent by the reactions they produce when a number of animals are injected.

Suckling lambs possess a relative resistance to bluetongue (18). Neitz (17) states, however, that lambs from susceptible ewes are also susceptible although they react to the virus less severely than do adult sheep; whereas immune ewes transfer passive immunity to their lambs via antibodies in the colostrum and milk to confer protection for periods varying from 4 to 68 days. Neitz believes that lambs may be successfully immunized after the age of two months, and that such immunity is probably good for at least 285 days.

Bluetongue may affect cattle as well as sheep. Spreull (9) reported that calves carried the virus in their blood for at least 21 days following injection of infected tissues; and Bekker, de Kock and Quinlan (7) and de Kock, du Toit and Neitz (19) found that cattle could contract bluetongue under natural conditions. The disease was called "seerbeck" or "sore-mouth" by the farmers. Terms such as "ulcerative stomatitis" and "pseudo foot-and-mouth disease" were likewise used to describe the condition. Cattle are, however, much more resistant to bluetongue than are sheep. The incidence is relatively low, outbreaks are sporadic, and while severe clinical symptoms occasionally develop, the course of the disease is milder than in sheep.

Adult cattle injected with the blood of naturally infected bovines show a slight reaction compared to the severe symptoms sometimes resulting from infection in the field. Inoculation of infected bovine blood, however, almost always gives rise to acute infection in sheep. To a lesser degree, it produces reactions in calves.

Goats usually have been regarded as resistant to the disease under natural conditions (1, 9, 20). However, in the 1950–1951 epidemic of bluetongue in Israel in a locality where most of the cows were affected, two Saanen goats were found with swollen lips, hyperemia of the oral mucosa and marked salivation (21). Furthermore, Spreull (9) found that although neither fever nor other symptoms occurred in inoculated goats, their blood contained virus from as early as the fifth day to as late as the 20th in some cases. The virus was transmitted for ten serial passages in goats without apparently enhancing or modifying the disease for sheep.

Neitz (22) investigated the possibility that wild game might act as natural reservoirs of the virus. Two blesbucks (*Damaliscus albifrons*) were each injected subcutaneously with 5 ml of blood from a sheep infected with bluetongue. Blesbuck no. 1 showed no visible bluetongue lesions at any time. It died 45 days after inoculation, not from bluetongue but from heavy worm infestation. Sheep subinoculated from the blesbuck on the eighth and tenth days developed typical but mild clinical symptoms of bluetongue following a five day incubation period. The sheep inoculated on the 14th day had a febrile reaction followed by development of lesions on the lips and feet. The incubation period was two days, and the sheep died. Sheep inoculated on the 20th, 24th and 40th days remained well and were not immune on subsequent challenge. Blesbuck no. 2 displayed no symptoms of bluetongue. Sheep subinoculated with its blood on the 17th day had a temperature rise, but no lesions, following an eight day incubation period. Sheep inoculated on the 40th day did not react and were not im-

mune when challenged. These results suggest that the blesbuck may serve as a reservoir of bluetongue. Other wild animals are possible carriers. Furthermore, as suggested by Bekker, de Kock and Quinlan (7) and de Kock, Van Heerden, du Toit and Neitz (23), cattle may serve as hosts in the transmission of the disease to sheep via biting *Culicoides*.

LABORATORY ANIMALS

Suckling white mice are susceptible to bluetongue virus injected intracerebrally (24; and personal communications from R. A. Alexander, V. J. Cabasso and G. I. Roberts). Injected mice show evidence of encephalitis. They become lethargic, prostrate, and die on the third, fourth or fifth day, depending on the strain of virus inoculated. Van den Ende and his associates (24) showed that virus was present in the central nervous tissues of suckling mice in titers as high as $10^{-6.7}$ to $10^{-8.4}$ when titrated in the same host, whereas the virus content of the spleen was negligible. Mice older than four days, but less than 13 days of age, were found susceptible to intracerebral inoculation. All attempts of Van den Ende and his associates to produce fatal infection in mice older than 12 days failed, even though they used virus repeatedly passaged in 10 to 12 day old "adolescent" mice. Intracerebrally inoculated adult mice showed no signs of illness, but there was evidence of limited multiplication and persistence of the virus through at least 14 serial passages. The titer of virus in adult mice titrated in suckling mice never exceeded $10^{4.3}$ LD_{50} per gram of tissue. The first evidence of virus multiplication in either suckling or adult mouse brain tissue was found after 8 to 12 hours, which is apparently the time necessary for a single multiplication cycle of the virus (24). Suckling hamsters likewise have been found to be susceptible upon intracerebral inoculation with various strains of bluetongue virus (personal communication from V. J. Cabasso). As will be described in detail later, the various strains of bluetongue virus grow readily in the developing chicken embryo (3, 12, 13, 14, 15, 16, 25).

GEOGRAPHICAL DISTRIBUTION

For many years, bluetongue was considered as being solely an African disease. It exists in all parts of South Africa, Southwest Africa and both Rhodesias, and as far north as French Sudan in the west and Abyssinia in the east (26, 27). A disease occurred in the Nile Delta in 1909 which Piot-Bey (28) regarded as bluetongue; goats as well as sheep were affected, and lesions of the feet and intestinal tract were absent. The diagnosis could be considered debatable.

Bluetongue was first proved to occur outside of Africa in 1943–1944, when the Onderstepoort laboratories checked a strain of virus isolated in Cyprus. They not only confirmed the fact that the disease was bluetongue but also reported the strain to be the most pathogenic thus far tested. The Cyprus strain was attenuated at Onderstepoort by serial passage through developing chicken embryos, and the vaccine produced was successfully used to control outbreaks in Cyprus in 1946 and 1947 (29). At the time of the 1943 outbreak in Cyprus, the disease in milder form was apparently seen in Israel (Palestine). It may have been present in Israel for a number of years before its existence was verified by analysis of infected materials at Onderstepoort. The disease, identified by adequate laboratory procedures (21), reappeared in Palestine in 1950 in both cattle and sheep (30). The Turkish Ministry of Agriculture reported bluetongue in sheep in 1945, 1946 and 1947, following the probable introduction of the disease from Syria (29).

Bluetongue apparently was first recognized as a new disease entity in the United States in Texas in July, 1948 (1). Only about 20 affected sheep were made available for study at that time, and all attempts failed to isolate the causative agent and to transmit the infection experimentally. No cases were observed in 1949. In 1950, only two affected sheep were seen, and attempts at transmission again were unsuccessful. The disease reappeared in Texas in June, 1951, on a much larger scale. About 15 western counties of the state were involved, in an area with altitudes ranging from only two or three hundred feet to somewhat over 2,000 feet above sea level. For the most part the disease appeared sporadically, sometimes on widely scattered ranches, but enough adjacent ranches were affected to suggest an epizootic. Although large numbers of Angora goats were present in the region, none was known to have been affected (1). During 1952, bluetongue was diagnosed on only four ranches in Texas (25). Then, starting in mid-May, 1953, another epizootic of the disease developed and spread to cover the sheep raising regions in West

Texas known as the Edwards Plateau and the Trans Pecos. It is of interest that each of the three epizootics in 1948, 1951 and 1953 originated along the Rio Grande River, which is the southwestern boundary of the area as well as the international boundary between Mexico and the United States. All three epizootics in Texas began either in May or June and terminated in the autumn.

Bluetongue made its initial appearance in California in September, 1952, and spread practically throughout the sheep raising areas of the interior of the state by the end of November, involving flocks totaling 325,000 (2, 3). Some affected bands were found on adjacent ranches, but others were found on well isolated ranches. The disease was observed in feeder lambs, yearling ewes, mature ewes and rams. Morbidity varied from a high of 50 per cent in some flocks to about 10 per cent in others (2). The overall mortality rate was estimated at 5 per cent (3).

In addition to California and Texas, bluetongue now has been officially diagnosed in Arizona and has been reported in New Mexico and Utah (31). These five states have a total sheep population of 10,590,000 (32), or approximately one-third the sheep population of the United States. Thus, bluetongue presents a serious challenge to the livestock industry of the United States, particularly to the sheep and cattle ranchers, and this challenge must be met by all the knowledge and resourcefulness at our command.

FACTORS AFFECTING DISTRIBUTION

In Africa the disease is distinctly seasonal, reaching its peak during wet and warm summers and in those parts of the country having a high summer rainfall. The highest incidence of the disease always coincides with the wettest months: February, March and April. When there have been heavy rains in the spring and early summer, the first cases of bluetongue may break out as early as December or even November. The disease is rare during the coldest months.

Bluetongue is consistently most prevalent in low-lying areas such as valleys, rivers, creeks and marshlands, and generally afflicts sheep exposed to the weather during the evening, night and early morning hours. Infection must take place during the cooler parts of the day or at night because areas considered unsafe for sheep at night are safe for sheep left in the open only during the daylight hours. The disease may be curtailed or prevented by moving animals to hilly parts of the same farm for the night, particularly if the animals are kept in closed stables. The absolute altitude above sea-level does not influence the incidence of the disease; the relative local elevation of the ground in comparison to the surrounding parts is the important factor. Thus, in Africa marshy areas 6,000 feet above sea-level have suffered outbreaks as have regions in Texas 2,000 feet above sea-level (1).

NATURAL TRANSMISSION

The disease is transmitted only by the injection of infected blood or other virus-bearing tissues. This fact, added to the observations already mentioned on increased incidence in wet weather and following night exposure in damp areas, supports the conclusion that a nocturnal biting arthropod must be the vector. Decreased incidence of the disease following the use of arthropod repellents in dipping vats lends further support to this theory.

Nieschulz, Bedford and du Toit (33) investigated several species of mosquitoes, including *Aedes caballus*, *A. lineatopennis*, *A. hirsutus*, *A. vittatus*, *A. dentatus* and *A. punctothoracis*, to see if they could transmit the disease. The mosquitoes were allowed to feed on infected sheep. Some of the insects were then made into suspensions which were injected into susceptible sheep; others were allowed to re-feed on similar animals. In only two experiments were positive results obtained, both with suspensions of *Aedes lineatopennis*. The findings indicated that this species might be a natural transmitter of the disease, but did not permit a decision as to whether it is an important or only an accidental or occasional vector. From an epizootiological point of view, *A. lineatopennis* appears to be a good possibility: it breeds extensively or not, depending on the amount of summer rainfall, in temporary water holes in low-lying areas well covered with grass. After breeding, the adults remain for quite some time at or near the breeding places.

In further studies, R. M. du Toit (34) showed that arthropods of the genus *Culicoides* (gnats, midges—sometimes called sandflies, punkies or no-see-ums) were natural transmitting agents of bluetongue. Three sheep injected with a suspension of *Culicoides* caught in the wild state de-

veloped typical bluetongue symptoms, and one died. The two survivors later proved to be immune. In another experiment, *Culicoides* were allowed to feed on an infected sheep and ten days later on a susceptible sheep. This sheep developed a typical bluetongue reaction.

Fiedler (35) identified 22 species of biting midges of the genus *Culicoides* in the southern part of Africa, three of which had never been reported previously. Fiedler states that these midges went unnoticed because they practically never bite man. The extreme dryness of most parts of Africa restricts breeding places for midges so that they do not generally constitute an annoyance even to domestic stock.

Recently in the United States, Price and Hardy (25) reported the production of clinical bluetongue in stabled sheep seven days after injections of a broth emulsion of *Culicoides variipennis*. The specimens of *Culicoides* were collected in a New Jersey-type mosquito trap near the Sonora, Texas, laboratory. A similar transmission trial carried out in sheep on another occasion resulted in a febrile response (maximum, 105.4 F) lasting from the seventh to twelfth day, and buccal hyperemia. *Culicoides variipennis* was by far the predominating species found at the time these experiments were made.

ETIOLOGY

Theiler (10) was the first to show that the infecting agent of bluetongue was a filterable virus. The virus is present in the blood, blood serum, tissue fluids, spleen and other blood-bearing organs of the affected animal. The serum is almost as infectious as the whole blood, showing that the virus is not confined to the cellular components. Usually virus is present in readily detectable amounts only during the acute febrile phase. By the time animals are noticeably sick, subinoculation of blood into susceptible sheep by the subcutaneous route produces a fever of only up to 105 F lasting about 48 hours, with none of the clinical signs of bluetongue. If further subinoculations are made, however, at this stage, the severe clinical picture will be produced. Alexander states, "failure to appreciate this rapid disappearance of virus from the blood before the onset of easily detectable symptoms has been the reason for failure to arrive at a correct diagnosis of the cause of mortality on many occasions" (14). Recognition of this fact led to the adoption in Africa of a routine diagnostic procedure in suspected cases of bluetongue. Blood is collected from the suspected animal as soon as possible. Five to 10 ml of this blood is injected intravenously into a susceptible sheep and, regardless of whether it produces a febrile reaction, is subinoculated into a second susceptible sheep seven to nine days later. A negative diagnosis is made only when the third subinoculated passage of blood fails to elicit symptoms. P. J. du Toit (36) showed that the bluetongue virus remains in the body of a recovered sheep for at least four months and possibly longer. He suggested that the virus might persist in the tissues of certain sheep until the following year, to be picked up again by a transmitting insect. However, a host other than sheep may be a reservoir for the virus in nature.

Bluetongue virus is quite stable and resistant. It retains its viability, even in decomposed blood, for extremely long periods. Theiler (10) used decomposed blood to obtain a bacteria-free filtrate which proved to be infectious when injected into sheep.

Neitz (17) carried out cross-immunity studies with ten strains of bluetongue and proved that there are antigenically different strains of virus, some much more pathogenic than others. The Theiler, Veglia, Camp, Nelspoort and Cyprus strains were isolated from susceptible sheep; Mimosa Park and University Farm strains were isolated from sheep immune to the Veglia strain; Bekker and Byenespoort strains were isolated from cattle; and strain C43 was isolated from *Culicoides*. Neitz demonstrated that each strain produced a solid and durable immunity against itself, and that the inadequate protection afforded by the monovalent vaccine then in use resulted from the antigenic plurality of virus strains encountered in nature. A variable degree of common or basic immunity relationship was evidenced by modified reactions in sheep immunized with heterologous virus strains. There was no increase necessarily in the incubation period, and frequently the maximum temperature was just as high in the vaccinated sheep as in the nonvaccinated controls. However, the course of the disease was usually shortened. In addition, vaccinated sheep had less severe buccal and hoof lesions and a considerably shorter period of convalescence. Neitz (17) further found that repeated injections of a homologous strain of virus had no detectable effect upon the common or basic immunity. He

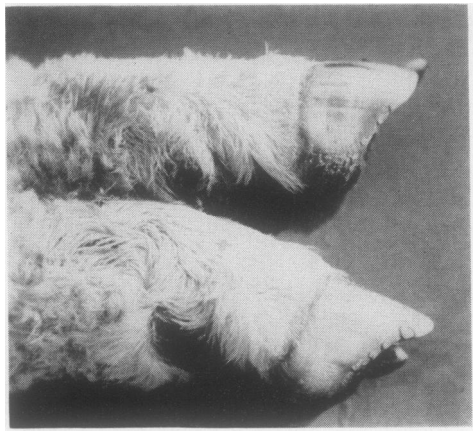

Figure 2. Coronary band lesions of a sheep suffering from bluetongue.

pointed out that a universally successful vaccine against bluetongue must be based on a full appreciation of the existence of virus strains different in antigenic structure as well as in degrees of invasiveness and pathogenicity. A polyvalent immunity, therefore, was the only safe immunity.

It should be noted that when Dr. R. A. Alexander, Director of Veterinary Services for the Union of South Africa, visited Texas early in 1953, he stated that as many as three immunologically different strains of bluetongue probably were present in Texas since one of the specimens that had been preserved from the 1951 epizootic clearly showed three distinct zones of coronitis (25). Subsequently, Price and Hardy have observed that multiple zones of coronitis are not uncommon, and occasionally a ranchman reports that his flock apparently had recovered from bluetongue only to be affected again a week or so later (25).

Polson (37) determined the particle size of blue tongue virus by both ultrafiltration and ultracentrifugation methods. Filtration procedures with Elford gradocol collodion membranes showed virus derived from either infected sheeps' blood or chicken embryos to have a calculated particle size of 100 to 150 mμ. By the bisectable capillary centrifugation method (38), bluetongue virus derived from infected sheep spleen had a calculated particle size of 108 to 133 mμ, whereas that from infected chicken embryo suspensions was 128 mμ.

CLINICAL CHARACTERISTICS

The disease in sheep. The susceptibility of individual sheep varies within wide limits, and the lesions in animals injected with a mild strain of bluetongue may be as severe as those in sheep injected with a virulent strain. Following artificial infection, the incubation period generally varies from two to four days but may be as long as 15 to 18 days. The incubation period following natural infection has not been determined with certainty but is believed to be less than a week. As a rule, the first sign of infection is a rise in body temperature although there have been afebrile severe and even fatal cases. The average maximum temperature ranges between 105 and 106 F, but temperatures above and below are frequent. There can be an irregular febrile response without any apparent disturbance of the health of the animal. In another case, an animal with no fever will present typical signs of bluetongue. Moderate fever at the onset of the disease is, however, usual.

Theiler (39) divided the clinical symptoms into abortive, acute and subacute types. The abortive type, in which the only indication is a rise in body temperature, may escape notice, particularly under field conditions. de Kock, du Toit and Neitz (19) encountered this mild infection in their studies of cattle.

The acute form of the disease produces a thermal response at the onset lasting from five to six days. The first clinical signs are nasal discharge and salivation. The tongue and lips often have an involuntary movement, and the buccal mucosa becomes red. Marked buccal lesions are not manifest sooner than about 48 hours after the temperature rise. The nasal discharge is mucopurulent and even blood-stained. The nasal mucosa, intensely congested, may appear cyanotic. Often there is swelling and edema of the lips, the gums, the dental pad and the tongue. The saliva is frothy rather than stringy. There may be excoriation of the epithelium of the gums, the inner parts of the lips and cheeks, the tip of the tongue, the bars of the hard palate and the dental pad. The margins of the lips bleed easily, especially when handled. Blood-stained saliva accumulates. Later, putrefaction sets in, and the mouth emits an offensive odor. When the buccal lesions are extensive, raw sores or necrotic ulcers are seen on manipulation. These ulcers are frequently regular in shape, elongated and lenticular. The usual site is at the sides of the fixed part of the tongue opposite the cheek teeth. In most cases, they are seen only on postmortem. The tongue, edematous, is dark blue or purple with amber or yellow

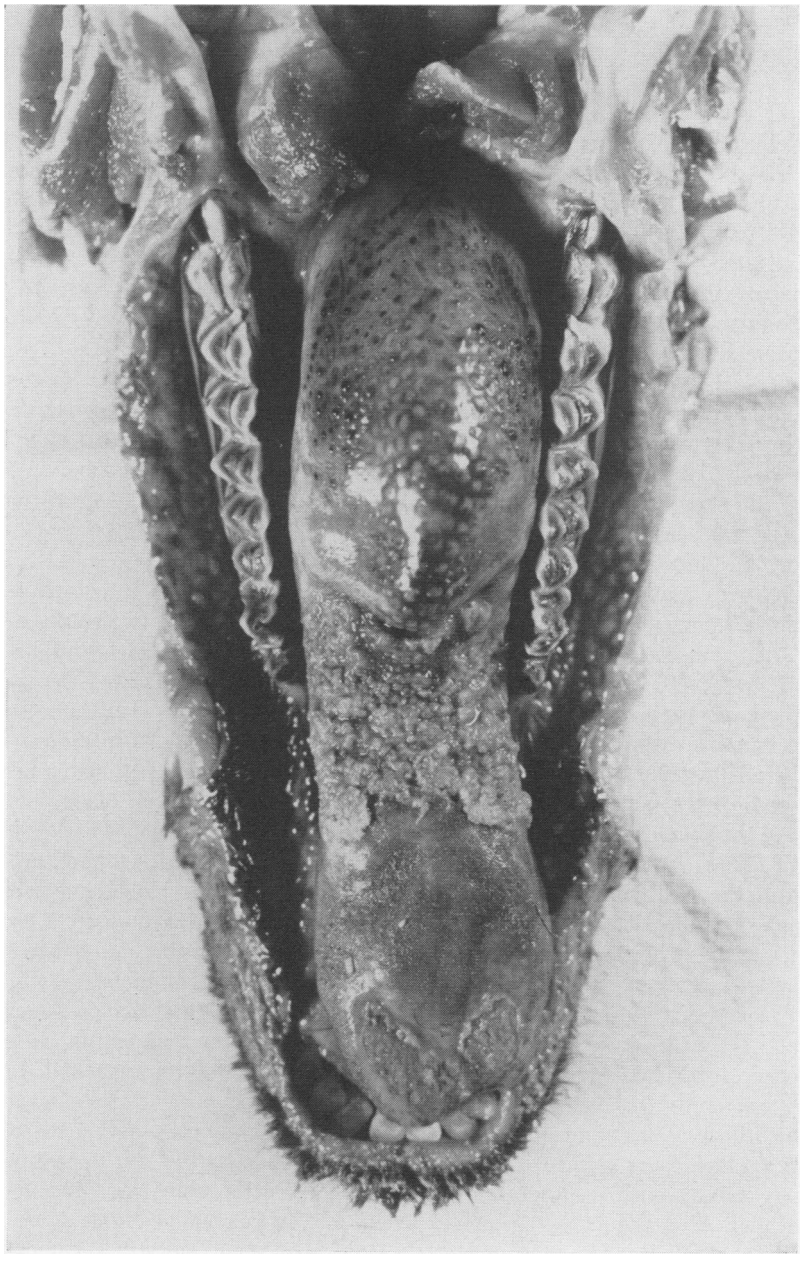

Figure 3. Tongue showing characteristic necrotizing ulcers.

patches along the sides. Swallowing is extremely difficult. The nasal discharge forms incrustations which partially block the nostrils. Respiration is often stertorous, depending on the extent of the nasal discharge, and animals may choke on their own secretions. The respiration may increase to 100 per minute; pneumonic symptoms are common. Thirst is often evident although animals may stand with mouths immersed in water merely to ease discomfort without making any effort to drink. Digestive disturbances are frequent, and diarrhea, often blood-stained, may occur. Blood may be passed in the feces in the absence of diarrhea.

In some animals, foot lesions form after three to seven days when the mouth lesions have begun to heal. A purplish discoloration of the skin often appears in the interdigital cleft. Occasionally the

pasterns and coronets swell, and may or may not be painful. In rare cases there may be exungulation.

Torticollis, or wry-neck, is associated with a relatively few cases of bluetongue. It may occur as early as the sixth day, but more usually around the 12th. It sets in suddenly. The head and neck twist to one side; the neck can be straightened manually without much difficulty, but when released it swings back again into the twisted position.

As a rule, there is a rapid and marked loss in condition. Sheep look dull and listless and show pronounced muscular weakness. They stand stiff and lame, with backs arched, or they may not be able to stand at all. The stiffness and muscular weakness apparently result from muscular lesions and may be seen independently of the coronitis. The state of debility, persisting in some cases for weeks, interferes with feeding. Some sheep, even though they have resumed feeding, continue to lose weight, their musculature wasting away rapidly.

Edema of the lower parts of the face and jaws may extend a variable distance down the neck. Quite often there is hyperemia of the skin of the muzzle, lips, base of the horns, ears, axilla and groin, or even of the skin of the whole body. The vulva and vagina are sometimes deeply congested. The nutrition of the wool suffers, and tufts of wool can be pulled out or parts of the fleece may be cast. Food may be regurgitated and expelled through the nostrils, especially if the animal has had access to plenty of water. Pneumonia may then ensue.

In fatal cases, death may take place from one to six days after the appearance of visible symptoms, but some animals linger on much longer. Animals which have seemed to recover completely may subsequently collapse and die three weeks or more after the first symptom.

The subacute type includes those cases in which the clinical signs are not severe, but in which there is great emaciation, prolonged weakness, occasionally torticollis, and an extended convalescent period.

Neitz and Riemerschmid (11) carried out experiments on shorn and unshorn sheep during four seasons of the year which demonstrated conclusively that both temperature reactions and clinical symptoms were greatly pronounced in sheep exposed to the sun. The detrimental influence of sunshine was evident in both naturally infected and vaccinated sheep. Bronchopneumonia developed, as well as multiple hemorrhages and muscular degeneration. To prevent such complications, care must be taken to vaccinate in the cool months of the year and to provide shade during the immunization period.

The disease in cattle. During the intensive campaign begun in 1932–1933 to combat foot-and-mouth disease in South Africa, Bekker, de Kock and Quinlan (7) reported a previously undescribed stomatitic disease of cattle which appeared in localities widely scattered over the Union. They believed its etiology was the well-known bluetongue of sheep. Unlike sheep, cattle did not usually show the blue or cyanotic tongue but did exhibit localized inflammation with necrosis of the buccal and nasal mucosa. All ages and breeds of cattle were susceptible, and all cases were found on the open range. No cases were observed in very young calves nor in cattle that were housed. The disease evidently did not spread by contact; even when diseased cows were confined with their calves in small stables and calves suckled the sore teats, there was no spread of infection. Perceptible reactions occurred in calves inoculated with infected blood, and virus could be recovered from them. They displayed a syndrome much like that seen in natural cases of the disease in cattle and bluetongue in sheep. Apparently, compared to sheep, only a few bovines are particularly susceptible, and only a small number exhibit alarming symptoms.

As in sheep, the first sign of illness was a febrile response, with the temperature dropping as soon as the mouth eruption appeared. In many cases where fever was the sole response, the disease was probably entirely missed. Sometimes a reduction in milk yield was the first indication to a farmer that his cattle were sick. The most seriously affected cattle remained lying down. Those which stood were lame and stiff and moved with difficulty when prodded. They made little attempt to feed, and rumination was suppressed. They often made chewing movements and ground their teeth.

In later studies, de Kock, du Toit and Neitz (19) recovered bluetongue virus from laboratory cattle "exposed on the veld at Tzaneen". However, these cattle showed no evidence of bluetongue as described by Bekker, de Kock and Quinlan (7): *e.g.*, lesions of buccal and nasal cavities, teat and udder involvement, skin changes

and foot lesions. The Tzaneen strain proved capable of "breaking the immunity" of sheep immunized with the Onderstepoort vaccine, even though sheep experimentally infected with this virus did not have typical bluetongue symptoms and none died. The mildness of the reactions of vaccinated sheep showed that the Onderstepoort vaccine conferred some basal immunity against the Tzaneen strain.

Mason and Neitz (40) failed to produce a recognizable disease in cattle by injecting bluetongue-infected sheep or cattle blood. A local lesion was produced in calves only when the buccal mucous membrane was scarified. Mouth lesions did not follow intranasal, subcutaneous or intravenous inoculations. The authors concluded that bluetongue virus brings about only an inapparent disease in cattle.

COURSE OF INFECTION

The prognosis is generally favorable in mild cases. Affected sheep may discontinue feeding for a few days, which naturally causes some loss in condition. Usually, recovery is rapid and uneventful. In severe cases, however, the prognosis may be uncertain. Animals which appear but slightly affected, or to have recovered completely, suddenly collapse and die. On the other hand, sheep which have had severe symptoms for a number of days may recover completely. Sore mouths make feeding difficult and painful, and the animals grow weak and emaciated. Deep-seated necrotic ulcers in the mouth may persist for considerable lengths of time. The loss in body condition and the debility cannot be attributed to oral lesions alone; as will be noted later, there is good evidence that the virus acts so as to depress the general nutrition or functioning of the body. Another possibility is that the persistence of the virus in the body following apparent recovery delays the return to good health. Convalescence is always slow, and particularly so in sheep with severe dermatitis and coronitis. Lambs take a long time to recover; they never thrive, are hypersusceptible to worm infestation, and remain runts.

Mortality varies from 5 to 30 per cent, with the loss much greater as a rule in young sheep than in matured animals, and in cases involving diarrhea. Even acutely sick animals seldom die before a lapse of eight to ten days following the first symptom.

The loss to the farmer may be great. Usually the fleece is much reduced in value because of break in the staple, or is actually cast and a breeding season is lost. Reconditioning a flock that has suffered from bluetongue may require weeks or even months. Debilitated sheep may be unable to withstand the rigors of winter, thus adding to the farmer's losses.

PATHOLOGY

Pathologic studies of experimental as well as natural bluetongue infections show changes mainly in the vascular system and the skeletal musculature. The lesions of bluetongue in sheep vary greatly in degree according to: (a) the strain of virus infecting the animal, (b) the individual and breed susceptibility of the animal, (c) the environmental conditions to which the animal is exposed, such as atmospheric conditions, quantity of exercise, and whether the animal has been shorn or not. A feature of the disease is that severity of lesions bears little, if any, relation to the height of thermal reaction (41).

The more important changes which characterize bluetongue in sheep, and from which the disease derives its name, are seen in and around the mouth. They are: diffuse and localized hyperemia, cyanosis, edema, multiple hemorrhages, swelling and excoriation of surface epithelium, and erosions and necrotic ulcerations of the mucous membranes. The tongue is sometimes gangrenous. The patchy hyperemia of the skin leads to scattered exanthematous areas and localized dermatitis. Sometimes there are abrasions on the exposed surfaces of the body. Bekker and his associates (7) described these lesions in detail.

The subcutis may show a few diffused red areas or spots; these are dark red, gelatinous areas extending at times from the subcutis into the muscles of the neck, shoulders and back. Colorless or reddish serous fluid usually infiltrates the intramuscular connective tissues and fasciae. Frequently small hemorrhages, one-half cm or less, are scattered throughout the musculature. The smaller hemorrhages form streaks or spots and may be overlooked. They are most readily seen when a thin strip of muscle is held to the light. Microscopic examination reveals many capillary hemorrhages in the muscle, where a diffuse cloudiness or opacity, usually quite localized, sometimes exists. Gross hemorrhages are not always present. Neitz and Riemerschmid (11), noted the presence of intramuscular hemorrhages

and muscular degeneration. Thomas and Neitz (41) later further described these lesions as well as those affecting the subcutaneous and intramuscular tissues.

Hyperemia is also found in the mucous membranes of the rumen, the folds of the abomasum and intestines, the liver, heart, kidneys and coronet. Multiple hemorrhages varying in size may occur in the small intestine, myocardium, epicardium, endocardium, and less often in the respiratory mucosa, bladder and urethra.

Microscopically, the sarcoplasm of the affected muscle fibers shows irregular swelling or bulging; the affected fibers are seen to be rigid, retracted or distorted among the normally wavy fibers. The sarcoplasm may be gathered together to form a bulge, leaving a length of empty tubular membrane (sarcolemma) nearby. The cross striations may be lost early (hyalinization) or persist until the sarcoplasm disintegrates and becomes phagocytosed. Early pyknosis of the sarcolemma may be seen, but rarely proceeds to complete necrosis (41). These findings help explain the weakness and debility, the severe emaciation, the torticollis and the prolonged convalescence. Recovery must await the regeneration of the sarcoplasm, and this process takes a long time (14).

A leucopenia and anemia may be discovered. In comparative studies on the blood of healthy and bluetongue-infected sheep, Graf (42) found no change in the concentrations of total creatinine N (nitrogen), amino acid N and uric acid N. There was a distinct increase in sugar, nonprotein N and urea N in both laked and unlaked blood. The hemoglobin level decreased in two sheep with severe reactions, and there was a corresponding decrease in total N in these cases.

DIAGNOSIS

A number of factors in addition to clinical symptoms and pathology enter into the diagnosis of bluetongue: these are the epizootiological features, locality and seasonal occurrence. Usually the disease is spotty in distribution and affects few animals at a time. The most important signs in sheep are the blue tongue and superficial localized inflammation and necrosis of the buccal mucosa, muzzle and external nares. The temperature rise is of little value since it has generally disappeared by the time mouth lesions develop sufficiently to attract attention to the animal. Coronitis, if present, develops when the mouth lesions are healing.

Cattle may also show swelling of the lower extremities and some hyperemia of the skin. Necrosis of the epidermis of the udder and teats in cows may be followed by scab formation. Dermatitis associated with scab formation may be present in other parts of the body. Cattle seldom display severe symptoms.

Complement-fixation tests. Van den Ende, Linder and Kaschula (24) have reported the preparation of good complement-fixing antigens from brains of the suckling mouse and chicken embryos infected with the Cyprus strain of bluetongue virus. The antigens were prepared by acetone and ether extraction following the method of Casals (43). Van den Ende and his associates also found that "soluble antigen" present in the supernatant fluid of tissue extracts after centrifugation at speeds known to cause sedimentation of bluetongue virus, was apparently as effective as the virus itself in complement-fixation tests. V. J. Cabasso (personal communication) likewise has prepared satisfactory complement-fixing antigens from the brain tissues of suckling hamsters and suckling mice infected with various strains of bluetongue virus of both South African and American origin. It is believed that the complement-fixation test will prove to be of great help in differentiating bluetongue from other viral infections of sheep and cattle, as well as an aid in the serologic classification of the various isolates of bluetongue virus.

DIFFERENTIAL DIAGNOSIS

Foot-and-mouth disease. Bluetongue may be differentiated from foot-and-mouth disease by its restriction to certain areas, by its scattered and seasonal occurrence, by its failure to spread by direct contact, and by the fact that the source of infection cannot be determined. The lesions of the two diseases occur on the same parts of the body, but the characteristic vesicle of foot-and-mouth disease, with its frayed irregular border when ruptured, is not seen in bluetongue. The lesions of bluetongue are essentially those of a localized inflammation (with necrosis of the mucous membrane), and in cattle skin lesions are usually present. In the latter stages, the lesions presented by the two diseases are quite similar. However, foot-and-mouth disease can be readily excluded if susceptible sheep are inoculated with blood

from the cattle and develop characteristic bluetongue symptoms.

Sweating sickness. This disease has the same seasonal occurrence in South Africa as bluetongue, but it does not affect sheep. It is largely confined to calves and young bovines. Unlike bluetongue, sweating sickness has never been known to occur seriously in highland areas. Its distribution is limited to the subtropical regions of the country. Both bluetongue and sweating sickness produce hyperemia and necrosis of the buccal mucosa, but the moist condition of the skin, followed by excoriation of the cutaneous epithelium over large areas, is characteristic of sweating sickness only. Furthermore, unlike bluetongue, sweating sickness cannot be transmitted by blood inoculation.

Three day sickness. The lameness, stiffness and temporary paresis are early symptoms of three day sickness and are much more marked than in bluetongue. Coronitis, hyperemia and necrosis of the buccal mucosa are absent in three day sickness. Furthermore, three day sickness apparently does not infect sheep under natural conditions.

TREATMENT

Good nursing care is the only form of treatment available. The affected animal or animals should be placed in a good stable or shed, well sheltered from sunshine, rain and wind. During the time the mouth is inflamed and tender, quantities of soft foods should be fed. Flushing the mouth and nose repeatedly with a weak, nonirritant disinfectant, such as potassium permanganate, or applying lotions containing alum and glycerin is helpful. Occasionally it may be advisable to give small doses of a laxative—sugar, for instance. Good nursing care and protection of the animal from the weather greatly increase the chances of recovery.

PREVENTION

Bluetongue can be prevented by practicing certain prophylactic measures. Susceptible sheep should not be quartered in lowland or swampy areas, particularly during the summer rainy season. If at all possible, they should be kept in sheds located on high ground during the night. Infection diminishes if sheep are dipped periodically in vats containing insect repellents such as coal tar derivatives.

IMMUNIZATION

The fact that no chemotherapeutic agent is known to be of any value, coupled with the relatively low value of an individual sheep and the large number of animals exposed to infection, dictates that adequate control must depend upon a safe, efficient and cheap method of immunization.

Spreull's (9) was the first method for immunizing sheep. It consisted of the simultaneous use of living unattenuated virus (virulent blood) and immune serum. Theiler (10, 44) could not confirm Spreull's results. He developed a vaccine in accordance with his belief that during successive serial passages in sheep the virus gradually lost its virulence for that species. He recorded no deaths in sheep from the 10th to the 18th passages, whereas an 11 per cent mortality took place during the first ten serial passages. Theiler's vaccine (see footnote 2, page 239) was known as O.C.G. (oxalate-carbolic acid-glycerine). It was administered subcutaneously in a one ml dose. Seven hundred ml of blood could be drawn from each donor sheep, furnishing approximately 1,400 immunizing doses of vaccine. The diluted blood from a number of sheep was pooled and bottled in quantities of apout 33,000 doses of vaccine per batch. Initially the vaccine was stored in a cool basement, but in later years it was held in a refrigerated room at 6 C. Each batch of vaccine was tested in at least two sheep and was released only if a satisfactory febrile response was produced. No bacteriological sterility test nor estimation of virus titer in terms of immunizing doses was made.

Theiler's vaccine was put into field use in 1907. The mortality in nonvaccinated flocks of sheep that year was 11 per cent. The mortality resulting directly from vaccination was estimated at 0.4 per cent. and deaths due to natural infection among vaccinated sheep was calculated to be 0.1 per cent. Thus, the total mortality in vaccinated flocks was 0.5 per cent as compared to 11 per cent in nonvaccinated. No data are available on the incidence of and recovery from infection in either group.

In an attempt to utilize virus donor sheep more economically, P. J. du Toit (36) investigated the immunizing effect of higher dilutions of blood. He concluded that an equally good vaccine could be prepared with blood dilutions of 1:2 instead of 1:1. Thus, each donor sheep produced 2,100

doses of vaccine instead of the former 1,400. P. J. du Toit also substituted a new strain of virus (Veglia) for the twenty year old Theiler strain in fear that the Theiler strain might have become too attenuated to produce a good vaccine. The new Veglia strain vaccine was introduced for field use in 1927, with results comparable to those obtained with the original Theiler vaccine. Complaints continued concerning the severity of reactions following vaccination, particularly during hot summer months, and concerning the failure to produce a solid, long-lasting immunity. Economic factors enhanced the seriousness of the situation; the price of wool fell, decreasing the profit from Merino sheep farming, and more susceptible English breeds of sheep were raised for lamb and mutton production.

In 1938, a decision was made to reinvestigate the whole problem of bluetongue to see if a more satisfactory vaccine could be developed. The Theiler strain came into use again, the second Theiler series originating from an old bottle of the O.C.G. vaccine that had lain at room temperature behind a cupboard in the office of a government veterinary officer for at least 25 years. Bluetongue is indeed an amazingly stable virus.

Several new and highly important findings emerged from the renewed investigations. First, Neitz (17) demonstrated that Theiler was mistaken in thinking that bluetongue virus was modified by serial passage in sheep. Neitz's conclusion was supported by all other workers except P. J. du Toit, who had reported success in attenuating a strain by this technique (36). Second, Neitz showed that since a number of antigenically different bluetongue strains existed, reliable protection required the use of a polyvalent vaccine consisting of a number of selected virus strains known to have a wide range of antigenicity. Third, Alexander and his co-workers propagated various strains of bluetongue in the developing chicken embryo, and attenuated them by serial passage through chicken embryos.

Mason, Coles and Alexander (12) first used eggs laid by hens on a riboflavin deficient diet. After four passages in these eggs the virus grew readily in fertile eggs from hens on a normal diet. The virus was carried for 21 consecutive serial passages, using six day old embryos, the yolk sac route of inoculation, and a postinoculation temperature of 36 C. The infected embryos died within four to five days and were cherry red when dead or dying. Infected chicken embryo tissues contained 10^5 to 10^6 infecting doses for sheep per gram of tissue.

Subsequently, Alexander and his associates (13, 14, 15) confirmed and amplified the early studies. Thus, presumably any strain of bluetongue virus can be grown in the developing chicken embryo provided careful attention is paid to the temperature of incubation. The virus will multiply at temperatures up to 38 C. Optimal results for seed virus are obtained by injecting eight, nine or ten day old chicken embryos with 500 to 1,000 infecting doses of virus into the yolk sac, incubating for 24 hours at 35 C, transferring the eggs to a temperature of 32 C and harvesting all embryos, dead and alive, at the end of an additional 48 hours. For the mass production of vaccine, it is satisfactory to hold the inoculated eggs for the full three to four days at 33.5 C (private communications from Drs. R. A. Alexander, V. J. Cabasso and G. I. Roberts). The chicken embryo suspensions show virus titers of 10^5 to 10^6 per gram of tissue. Estimation of the virus content of chicken embryo adapted strains by titration and injection of 0.2 ml quantities in eight day old embryos incubated at 33.5 C corresponds closely with titrations of the same material injected subcutaneously in 2.0 ml amounts into susceptible Merino sheep.

The results of the chicken embryo studies in South Africa have been confirmed in all major respects by McKercher and his associates (3), who have grown at least two California bluetongue virus isolates in chicken embryos. Price and Hardy likewise have definitely established one Texas isolate (Sonora) of bluetongue virus in chicken embryos and apparently have isolated three additional strains which have been successfully carried through several chicken embryo passages. Two of the latter were isolated from field outbreaks of bluetongue in sheep, while the third was derived from a sheep artificially infected with *Culicoides variipennis* collected near the Sonora, Texas, laboratory (25).

Alexander and his co-workers succeeded in modifying virulent strains of bluetongue virus to a point where practically no clinical reaction was produced in sheep. Further, once a strain of virus had been attenuated by chicken embryo passage, its virulence was not restored or enhanced by eleven serial passages in sheep.

These facts made possible the present quadri-

valent living virus vaccine, which, according to Dr. R. A. Alexander (personal communication), is now prepared at Onderstepoort with chicken embryo seed virus from the following strains: 101st passage Cyprus; 60th passage Estantia; 56th passage Bloukop; and 30th passage of the original Theiler. The different virus strains are grown separately in chicken embryos, then pooled. The resultant vaccine contains approximately 250 LD_{50} chicken embryo doses of each strain per 1.0 ml dose for sheep. Both fluid and freeze-dried preparations have been issued in the past, but for practical and economic reasons the desiccated form will probably be distributed in the future. During 1953 the Onderstepoort laboratory produced a little more than 5 million doses of vaccine, and the anticipated need for 1954 is between eight and 12 million doses (R. A. Alexander, private communication).

The vaccine is administered subcutaneously in one or two ml amounts. Regardless of volume, however, each vaccinating dose should contain at least 250 chicken embryo infecting doses of virus for each strain in the vaccine. Sheep react slightly, if at all. Occasionally, a hypersusceptible sheep will develop some buccal hyperemia, slight coronitis, and dullness or listlessness about the ninth day after vaccination. Even though reaction is moderate, and sheep may be injected safely either before or after shearing, it may suffice to cause anestrus in ewes. Therefore, they should not be vaccinated within three weeks of mating. The best immunity results from annual revaccination at least one month before the usual time of outbreak of the disease.

The vaccine has no curative value, but immunity develops rapidly usually within ten days of vaccination. If an outbreak does occur, early vaccination will protect a great portion of the flock from infection; deaths and severe cases will diminish on or about the tenth day after vaccination. Care must be taken not to transfer pathogenic virus from sick to healthy sheep by contaminated needles and syringes in the vaccination procedure.

There is a period during which the vaccine will not immunize lambs. Alexander describes this problem as follows:

"Newly born lambs out of immune ewes possess a high degree of passive immunity which decreases fairly rapidly. The duration of this transient immunity is not known with any accuracy, but it probably lasts about three months. During a portion of this period the immunity is insufficient to protect against natural infection but is sufficient to neutralize the attenuated virus in the vaccine. Therefore, it is not possible to immunize lambs from immune ewes dropped in the spring or early summer before the onset of natural bluetongue. In other words, the effective control of bluetongue in young animals is an animal husbandry problem as well as an immunological problem, and in the bad bluetongue areas breeders must realize that early summer lambing is simply uneconomical" (14).

In conclusion, some comment should be made on the bluetongue virus isolated in California. Alexander (private communication) noted in preliminary experiments that the Mimosa Park strain gave complete protection against the California virus, as did the present South African quadrivalent vaccine. The California virus is believed to be not too highly pathogenic, yet sheep immunized with it have been protected apparently against infection with a mixture of Cyprus, Estantia and Mimosa Park strains; thus indicating the broad immunogenic spectrum of the California virus. At least four commercial laboratories now have been licensed by the United States Department of Agriculture to manufacture and distribute bluetongue vaccine, using two California strains of virus isolated by McKercher (3) and following Alexander's procedure. Other studies aimed particularly at the attainment of better diagnostic and strain classification methods are being pursued. Our knowledge in this area soon should be considerably increased.

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