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**BLUETONGUE.  
A REVIEW OF THE DISEASE**



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**BLUETONGUE.  
A REVIEW OF THE DISEASE<sup>1</sup>**

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## 1. DEFINITION OF THE DISEASE

Bluetongue (BT) is an infectious, noncontagious insect-borne viral disease of sheep and other ruminants (14).

## 2. VIRUS CHARACTERISTICS

### 2.1 Physical Characteristics

Bluetongue virus (BTV) belongs to the family *Reoviridae* and to the genus *Orbivirus* (31). It has a double-stranded ribonucleic acid (RNA). The virus particle is spherical and about 69 nm in diameter. The double-stranded RNA has 10 genome segments (31). The antigenic determinants are possibly carried by one protein for which only one of the segments provides the code (31).

The BTV core particle contains two major polypeptides, P<sub>3</sub> and P<sub>7</sub>, and is surrounded by an outer capsid layer that is composed of the two major polypeptides, P<sub>2</sub> and P<sub>5</sub>. The P<sub>2</sub> protein gives rise to type-specific antibodies (48, 50) and the gene segment 8 of BTV type 17 codes for the intergroup-specific polypeptide (36) (see also 2.2).

The BTV group specificity, as measured by a complement fixation test (CFT) or agar gel immunodiffusion test (AGID) is determined by the core protein P<sub>7</sub> (48, 54).

### 2.2 Serotypes

There are 23 or more serotypes (26, 92). It is not known how the different serotypes have arisen, but they probably emerged by antigenic drift. Reassortment has also occurred (54). During this century at least some strains have shown to be immunological stable.

Orbiviruses are characterized by complex serological inter-relationships (Table 1) (26, 92).

The BT group is formed by the common group antigen which can be demonstrated by serological tests, such as: CFT, AGID, and fluorescent antibody test. Specificity between or within groups is shown by neutralization test.

TABLE 1. Related Orbiviruses within the family *Reoviridae* (78).  
The bluetongue group (=Orbivirus group B)

Virus	Types	Distribution
Bluetongue	1-23 +?untyped	World-wide
Epizootic haemorrhagic disease of deer (EHD)	New Jersey EHDV <sub>1</sub> EHDV <sub>2</sub> Alberta (=Ibaraki) Australian and Nigerian isolates	North America Large part of Caribbean area Trinidad, Tobago, Guyana (37) West Africa Asia Australia (54)
Ibaraki	=EHD Alberta	Japan South East Asia
Eubenangee		Australia
Pata		Central Africa
Tilligerry		Australia

### 2.3 Stability

The virus is very stable and extraordinarily resistant to influences that destroy most other viruses quickly. It will withstand, for example, a considerable amount of putrefaction. The virus has been recovered unchanged by filtering highly decomposed blood (29). Blood and meat can remain infectious during months. Also dried blood is still infectious (38). Fresh blood, conserved in glycerine and kept at room temperature, was still infectious after 25 years! (96).

In washed erythrocytes stored at 4°C during 14 months, the virus still causes a delayed but typical BT clinical response in autografted sheep (59).

The virus is comparatively resistant to sodium hydroxide, sodium carbonate, ethylalcohol (29), ether and chloroform (13). The virus is sensitive to acids. It is inactivated by pH 6,4 for

10 min (13). When the pH in meat stays above 6.4, it will remain infectious for long periods. The virus is inactivated when kept at 60°C during 30 min (18). The particle is stable at 4°C and -70°C but loses infectivity rapidly when frozen at -20°C (78).

Effective disinfectants include acids, alkalis and iodophors.

### 3. HOST RANGE. SUSCEPTIBILITY

Bluetongue is primarily a disease of sheep and all breeds are susceptible although to variable degrees (14). The European mutton breeds are the most susceptible, followed by the Merinos. The Dorset Horn, for example, is more susceptible than the Merino. Indigenous breeds are the less susceptible. The most, if not all, indigenous African sheep are resistant to BT disease (42). However in Cyprus, local breeds were badly affected (80). Epizootics can be expected in European breeds.

In experimental infections of goats and sheep, the highest virus titer in the blood of goats was found to be a 100-fold lower than that in sheep (15). Komarov and Haig (1952) reported natural infection in Saanen goats in Israel, and Botija (1958) reported mild BT disease but no mortality in goats in Spain (mentioned in: 42).

The virus does not produce overt clinical disease in cattle in South Africa.

However, when epizootics were reported outside Africa, clinical signs were observed in cattle in Israel, Palestine, Syria, Portugal and Spain. In the USA, in some infected herds, approximately 5 per cent of the cattle show from mild to severe disease (41). In newly infected areas, the clinical symptoms of cattle resemble those of susceptible sheep (49).

Of the wild animals the white tailed deer (*Odocoileus virginianus*) is the most severely affected (78). Many species of antelopes are susceptible. In African antelopes the infection passes normally without clinical symptoms, although death, caused by BTV, occurred among Topi-antelopes in 1962 (38). Also a fatal case of BT in experimentally infected buffalo calves is described (15). In India, the prevalence rate of BTV infection in buffaloes is similar to the prevalence in cattle (94). Antibodies are found in pigs and camels (1).



Bluetongue is not transmissible to man. Insusceptible are: dog, cat, rabbit, ferret, guinea-pig, and single-hoofed animals.

#### 4. EPIZOOTIOLOGY

##### 4.1 Transmission

The virus is transmitted by midges belonging to the genus *Culicoides*. In this vector the virus undergoes a biological cycle (49). Pathological changes have been reported in the salivary glands of *Culicoides* (42).

In North America the main species of the *Culicoides* vector is *Culicoides variipennis*, that can transmit BTV from cattle to cattle, cattle to sheep, sheep to cattle and sheep to sheep (14).

There is some speculation with regard to the role of *Culicoides insignis* in the Caribbean area (53).

In Africa and the Middle East the major vector of BTV is *Culicoides imicola* (= *pallidipennis*) (61).

In Australia vectors are: *Culicoides fulvus*, *C. wadai*, *C. actoni* and *C. brevitarsis* (86).

Different geographic populations of the same vector-species differ in their competence to act as vectors (25).

Bluetongue virus has also been found in the following biting insects, which only transmit the virus mechanically: cattle lice, sheep lice, biting flies, ticks and sheep keds.

Oral or contact infection does not take place and experimental infection is done mostly by parenteral inoculation of infective blood or tissue suspensions.

It was not possible to determine the factors that control the spread of BTV in the Caribbean (26, 92), but it is remarkable that the same virus types have spread throughout this area within one year. The time of virus onset in each area is not yet known (26, 92).

##### 4.2 *Culicoides* spp.

The female midge attacks livestock. It requires at least one blood meal for the completion of the ovarian cycle (67).

Numerous factors determine the intensity of their attacks but they take a blood meal every 3-4 days (78). However, when the midge takes an infective blood meal, it remains infected for life, which can be as long as 70 days.

Their optimum environmental conditions are between 13-35°C. *Culicoides* is active from just before dusk to just after dawn (67) and is carried on winds. The insects can complete distances of 40-700 km in periods up to 20 hrs and can be transported at heights up to 1,500 meters. The midge takes  $10^{-4}$ - $10^{-5}$  ml blood/meal (78). A high virus titer is also needed for transmission. The virus multiplies in the midge. After this multiplication each midge can contain as much as  $10^5$ - $10^6$  infectious particles (31). Seven to ten days after the blood intake it is able to transmit (22, 78).

Transovarial transmission of BTV in *Culicoides* is unlikely (15).

#### 4.3 Virus Reservoir

The available data given on the virus persistence in the host are confusing: persistence-time of only 10 days in sheep (variable for every strain) (38), up to 50 days in sheep and 100 days or more in cattle. It appears that the isolation method is very important in this case. More sophisticated or appropriated methods, yield virus isolations for longer periods.

Different possibilities of the localization of the virus in the blood are mentioned in the literature. According to some authors the virus is free in the blood (18), while others found that the virus persists in the red or white blood cells. It seems that the virus is closely related to erythrocytes and/or white blood cells because of the following tests: embryonated chicken eggs (ECE) could be infected by the intravenous route, equally well with whole blood, plasma and buffy coat cells (33), and washed erythrocytes stored at 4°C remain infective as long as 14 months (59) (see also 10. Pathogenesis).

In the USA, cattle are considered the most important reservoir host of BTV. Cattle may form a winter reservoir of BTV (USA) (64). It is also possible that adult midges may overwinter in some areas.

White-tailed deer (USA) are presumed to be a short-term reservoir of BTV. Normally the disease is acute in this species and they show a short viremic period. Recently it was found that, also in this species, an inapparent form of BT can occur.

The question arises if deer also can serve as long-term reservoirs like cattle do, or that an avirulent strain of BT is circulating among deer (55).

For South Africa it was postulated that sheep are not essential for the continued survival of BTV, but merely function as accidental or indicator hosts (15).

The perpetuation of BTV within a population also is possible by transmission via semen or by transplacental infection of the fetus (58, 88). Infected bulls may shed BT virus in their semen. In bulls, infected for the first time, BTV was found in the semen nine days after infection. In literature, the data of detectable virus in semen varies between seven and 106 days after infection. Although the occurrence of BTV in semen is demonstrated, it appears to remain an occasional event (34). BTV is believed to be present in the semen as a result of infiltration of infected blood cells into the genital secretions. In certain circumstances it may be present within the germcell or spermatozoa. Abnormalities and virus-like particles have been described in spermatozoa from bulls, latently infected with BTV. Transmission through semen has been demonstrated by natural service and by artificial insemination (8, 63, 79). In the USA, a certification protocol has been developed to certify germplasm free of BTV (69).

Bluetongue virus can cross the endometrium as was shown that virus could be isolated from the blood of heifers which were infected experimentally by the uterine route (34). Also transplacentally virus transmission "in the other direction" is possible. Fetal infection apparently occurs shortly after acute viraemia in the dam (57). There is little known about the virus localization in the embryos (43), but apparently virus can be isolated from the blood of the fetus (18). *In vitro* exposure of bovine embryos to BTV has shown that the virus easily infects embryos (27). However, embryo transfer from cows with viraemia has a low risk of transmitting BTV. Vertical transmission of BTV during the first eight days of gestation by embryo transfer is unlikely, because the zona pellucida of these embryos prevents embryo-infection.

The recovery of embryos with the non-surgically method nearly always is accompanied by some degree of blood contamination and if the cow is viraemic virus can be isolated from the flushing medium. However, routine washing procedures of the embryos before transfer eliminates virus transmission (27).

In experiments with transferring embryos from viraemic donors into BT negative cows, none of the 40 recipients turned BT positive in the AGID-test, nor virus was recovered (7, 27). Also others concluded that embryo transfer is not associated with transmission of BTV (75) and that congenital transmission is not important and BTV usually behaves like a classical arbovirus (93).

#### 4.4 Virus Carriers

The carrier state has not been fully defined and latent carriers do not always have detectable antibodies (33).

Cattle known to harbor virus may be serologically negative to the standard tests (33). This lack of BT-antibodies and presence of BTV might be a characteristic of latent infection in certain cattle (comparable with mucosal disease where also often no serum neutralizing [SN] antibodies are formed [60]) (see also 12. Diagnosis).

Nothing is known about the site of BTV replication in latently infected cattle or in what form the virus is present in the animal, other than it is in close association with erythrocytes (58). In latent infected cattle, it often is difficult to isolate virus from the blood circulation, but about one hour after experimental exposure to *Culicoides*, animals will develop a detectable viraemia. The mechanism behind this virus stimulation is not known yet (100).

## 5. WORLD DISTRIBUTION

### 5.1 History

The first recording of the BT comes from South Africa, in 1876 (14), and the first description of the disease was given by Hutcheon, in 1881 (mentioned in 31).

The disease was recognized in Egypt, in 1907 (in Merino breeds), Kenya, in 1909 (specially in sheep in the highlands), and in West Africa from 1927 (in imported European or Merino breeds).

Bluetongue was not identified outside Africa until the virus was isolated in Cyprus, in 1943, although the disease has been observed on the island since 1924 (77). Another severe outbreak occurred in Cyprus in 1977 (BTV type 4), when local Cyprus breeds as well as imported Awassi and Chios breeds were equally affected.

From 1943-1949, the BTV was active in Palestine, Turkey and Syria.

In Iraq local breeds have been observed with BT.

In Turkey and Israel, the disease was seen in Merino and European breeds. An extensive outbreak in cattle and sheep took place in Israel in 1950.

In 1956, local Merino-type of sheep were affected in Morocco. Also in 1956, a severe epidemic occurred in Spain and Portugal, caused by virus type 10 (25); 81,000 animals (cattle and sheep-Merino breeds) diseased with a mortality of 6.3 per cent. The disease disappeared after a few years. *Culicoides imicola*, the major vector of BTV in Africa, was identified for the first time in Spain in May 1982. It could have been the vector that brought the virus from Africa (Morocco) to the Iberian Peninsula in 1956 (61).

Egypt had more outbreaks in 1963-66 and 1969-70 (first outbreak in 1907) (77).

In the USA the disease was recognized in 1947 (Texas), but the first virus isolation was only made in 1952. The virus spread from Texas to California and from there further eastward (62).

Brazil reported serological evidence of infection in livestock for the first time in 1979 (4, 26, 27, 92).

In Chile, the first report of antibody for BTV dates from 1982. The AGID test indicated a prevalence of 20 per cent of BT antibodies among the cattle of the "Region de Los Lagos" (91).

In Australia, the first virus isolations were made from insects in October 1977. (23, 52, 95) (serotype 20), during a routine survey of insect viruses. Cattle in this region (the NW of Australia) appeared to have antibodies against BTV, but the clinical disease was never seen (3). Sheep were experimentally infected with this strain and showed only mild clinical symptoms (10). It is presumed that the virus entered the country by crossing the sea between Papua New Guinee and Australia, which is a distance of only 160 km.

During a country-wide survey, in December 1978, 200,000 blood samples were taken from sheep, cattle and other ruminants. The first results were all negative. However, in the first

seven months of 1981, when seven sentinel cattle herds in different locations in Northern Territory were monitored for arbovirus, BTV antibodies were found in six herds. Subsequently, four BTV isolations were made from blood samples of healthy cattle in BHK 21 tube monolayers (21). Later publications mention sero-conversion to BTV in almost every state of Australia (12).

In Turkey – after a free period of nine months – new cases were reported in October and November 1978. In four provinces, 606 sheep were affected of which 148 died. After the first outbreaks the disease spread in a milder way. The causal agent was identified as BTV type 4. Since November 1978, the disease was controlled and as a preventive measure all the sheep in these areas were vaccinated in summer 1979 (3).

In 1979, 482 sheep were infected and 100 sheep died. There was only one outbreak of BT in 1980 with no deaths and 40 affected sheep (68).

Bluetongue in cattle: BT was first implicated as a disease of cattle in 1934 in South Africa (39, 52) and is further reported in Israel, Spain, Portugal, USA and possibly in Cyprus.

## 5.2 Actual Situation

While vector and vertebrate hosts exist nearly everywhere in the world, the virus seems able to establish itself north and south from the equator to only somewhat temperate climatic limits, perhaps because of some quantitative climatic limitations in the vectors (93). It is hypothesized that BTV does not replicate in insects where the metabolic rate is reduced below a certain level at which the insect can survive, but is unable to produce viable BTV (64).

It is important to distinguish between only serological confirmed prevalence of the BTV (antibodies) or the diagnosis of clinical BT disease followed by virus isolation.

Africa - Virus and antibodies in most of the countries: Morocco, Sudan (5), Tchad, Nigeria, C.A.R., Kenya, Tanzania, South Africa, Zimbabwe. Almost all the serotypes are present in South Africa; also in West Africa. For example, Nigeria: 1-16, 18, 19, 20 and 22 (40).

Europe - At the end of 1979 an outbreak occurred on the Isle of Lesbos, Greece (type 4). This island is situated at 10 km from the coast of Turkey (97). In May 1982 *C. imicola* was identified for the first time in Spain. The presence of *C. imicola*

should now be established in other countries of the European Economic Community (EEC), at least as far as 40°N, e.g. Sardinia, Sicily, southern Italy and Greece (see for distribution throughout Europe ref. 56).

If infected midges can reach these countries by wind from North Africa, they could set up cycles of infection in the local midge populations. Then epidemics similar to those experienced in Spain and Portugal in 1956 could occur (61).

West Asia - Virus and antibodies in Turkey, Syria, Lebanon, Cyprus, Jordan, Iraq, Iran, Israel, Yemen, Oman. Types 1, 3, 4, 10, 12 and 16 (40).

Russia - The country seems to be free, but an inactivated vaccine has been developed. Regularly serum surveys of BT antibodies are made along the borders of the country. Import of susceptible animals from infected countries is prohibited (83).

India sub-continent - Virus and antibodies in India (1961) and Pakistan (1959). BT is endemic in local animals. Disease has been seen only in imported sheep of the American and Australian breeds.

#### The Americas:

USA - Virus isolates present in the USA are 2, 10, 11, 13 and 17. In May 25, 1983, serotype 2 virus was isolated from blood taken from a herd of cattle in Florida in September-October, 1982 (Callis, J.J. personal communication, and 28). Only five of the 23 serotypes are known to infect ruminant livestock (26, 92). All breeds of sheep seem equally susceptible in the USA. The BTV is frequently isolated in the USA and antibodies have been found in most states. In California and Mississippi 31 per cent of sheep and cattle are serologically positive for antibodies (62). In 2.8 per cent of Louisiana goats, BT antibodies were found (20).

Canada - The correct ecological circumstances do not exist in this country. However, in 1976, 220 cattle, contacts of former imports in 1975 from the USA, were destroyed. In the following three years no further seropositive animals were found in spite of testing large numbers of animals (44).

Central America, Caribbean, South America - The disease itself has never been seen or confirmed by virus isolation from ruminants nor from arthropods.

A high percentage of sheep, cattle and goats have precipitating antibodies against BT. In these three species in the Caribbean area about 70 per cent are positive by the AGID test (26, 92).

Serotypes: 1, 2, 4, 14 and 15 in St. Vincent; 1, 2, 5, 6, 7 and 10 in Barbados.

In 1981 or first half of 1982, types 6, 14 and 17 were prevalent in the Caribbean (26, 53, 92).

Antibodies have been found in cattle in Puerto Rico and the U.S. Virgin Islands (26, 92).

Antibodies have been found in Mexico, Colombia, Venezuela, Ecuador, Brazil, Peru, Paraguay, Guyana, Surinam and French Guiana.

In Chile the first report of BT antibody dates from 1982, and in Brazil the first serological evidence for the virus was found in 1979 (type 4). During a survey in the state of Rio de Janeiro, Brazil, 40 per cent of 550 cattle sera were positive to the agar gel precipitation test (AGPT). Also antibodies were found in the states of Amazonas, Pará, Minas Gerais and São Paulo and in the Territories of Roraima and Amapá (4, 11).

During a serological survey in Argentina (specially the northern part of the country), no positive samples were found. It is thought that Argentina is beyond the ecological zone where the disease cycle can exist (Schudel, A.A., INTA-Buenos Aires, 1984, personal communication).

South-East Asia and Australia - BTV types 20, 21, 1 and CSIRO 154 (= related to serotype 6 [24]) isolated [34, 95]).

Antibodies: Australia, Papua-New Guinea, Indonesia and Malaysia.

In Australia BTV causes little clinical concern (26,92) (see 5.1 History).

Japan - BTV 4, 12 and 20 exist in Japan (66).



6. SEASON OF ONSET OF THE DISEASE (78)

South Africa - January, March.

Egypt - April, June, July.

Israel, Cyprus and Turkey - July to December.

USA - June in Texas; later in the year in other parts of the country. Abortion and masked infections during winter and spring (41).

Portugal and Spain - Occurred in the first year (1956) from June to November. Following years, from April onwards.

Countries near the Equator - Most months of the year. In Brazil, seroconversion in one dairy herd was observed in the state of São Paulo during the early months of the year (Sutmoller, P. & Alonso Fernández, A., personal communications).

Peaks of disease in:

Kenya - February, June, July, October  
West Africa - September, October  
India - September, October

7. EPIDEMIC AND ENDEMIC BEHAVIOR IN THE AMERICAS (78).  
SEASONAL EFFECTS

7.1 Endemic Areas

In the Amazon region and the tropical forests of Central America the vector is present all year round and a continuous cycle of BTV infection can be maintained in *Culicoides* and local ruminants. After the loss of maternal antibodies the young animals become infected but local animals do not show the disease.

In other regions of Central America, there is a wet and a dry season. During the dry season, the number of *Culicoides* diminish, but there remain sufficient to maintain the virus during this period. The local animals do not show clinical disease, but introduced sheep do.

In California, with cool winters, BTV survives the winter months in adult midges or in cattle, sheep or goats. Disease may occur, depending on the breed of sheep.

## 7.2 Epidemic Areas

In parts of the USA where the winter is cool, the BTV may be introduced by flies carried on the wind, or by movement of animals. It may persist for one or two years, disappear and be reintroduced again. Disease is usually seen in sheep.

In Canada BT is rarely introduced. Disease occurs in the sheep. After the vectors diminish due to the long winter, the disease dies out.

Remark: Multiple serotype infections are possible in cattle and sheep (74).

## 8. CLINICAL SYMPTOMS

Clinical symptoms in sheep vary from mild to severe, depending on the strain of virus concerned, the breed of the sheep, the environment, and the epidemiology of the disease in the country (78).

The pathogenicity of virus strains differs markedly (14) but the severity of the disease is not predictable for different virus types (30). It is possible that BT occurs in sheep without causing clinical symptoms (76, USA).

Among sheep, all the ages are affected, except the lambs of immune sheep. In endemic areas, the highest mortality occurs among the one-year old sheep (38).

### 8.1 Characterization of the Disease

Seasonal disease of sheep with congestion of the buccal and nasal mucous membranes and of the coronary band of the hooves and stiffness by muscle degeneration.

### 8.2 Symptoms in Fully Susceptible Sheep

Incubation period - About one week (38).  
Australian data: 1-7 days (3).

The incubation period following artificial infection of sheep may vary from 2-15 days with an average of about 4-6 days.

First: Increased respiration rate occurs shortly before or at the onset of the fever and is usually associated with the peak viraemia. Peak fever usually occurs about one day after

the onset of respiratory distress (63). The rise of body temperature (T) is usually between 40.6-41.7°C. The degree of fever does not predict the severeness of the symptoms (18). During about 48 hs the T keeps on at this level, thereafter the T fluctuates between 39°C and 40°C during 6-8 days (49). The sheep refuse to eat, are licking the lips and are making strange movements with their tongue.

After 24-48 hs: Nasal discharge and salivation appear. This nasal fluid does not contain virus (96). At first, the fluid is watery, thereafter mucous and later it dries forming crusts on the nose. The nasal mucosa is congested with possible ulceration. In that case the nasal discharge will turn bloody. The lips bleed easily when touched.

After 3-5 days: Mouth lesions appear. The mucosa of the mouth is congested and is often cyanotic. Superficial ulcers are formed quickly. Sheep may die during the acute stage of the disease with massive haemorrhages in the heart (63).

After 5-8 days: Necrosis is formed with ulcers (white debris), causing a foul smell from the mouth. The saliva is mixed with blood. The ulcers are irregular shaped, with a diameter of 2-4 cm and with haemorrhagic basis. The recovery from these ulcers is slow and takes place under a difteric membrane. This difteric necrosis is caused by secondary bacterial infection (49).

In only a small percentage of sheep the characteristic purple-blue coloration of the tongue can be seen (18). However, the Australian literature reports that often a swollen bluish tongue can be observed (3).

Lenticular necrotic ulcers which may develop on the lateral aspects of the tongue are pathognomonic for BT. These lesions cause the tongue to become swollen and purple in color. These lesions are unlike those in any other disease of sheep. Sometimes there exists oedema on the head, ears or mandibula, sometimes below the belly.

At the end of the febrile reaction: Lameness or stiffness often occur. In mild outbreaks these sometimes are the only symptoms. The lameness is caused by an inflammation of the coronary band, appearing as a red or purple ribbon, specially pronounced on the bulbs and more frequently observed in the hind feet. This band will grow down with the horn of the hoof. After 10-12 weeks the band disappears.

The hooves are very painful. The animals do not move, go on their knees or stand still with kyphosis. The stiffness is caused by a degeneration of the skeletal muscles (63). Sometimes an animal will get sick only after the fever is gone (stiff, bent back, apathic).

Excoriations can occur on the legs when the sunshine is very strong, there is no pigment and the wool is short. A certain degree of sunlight hypersensibility occurs. Therefore the disease is more severe in recently shorn sheep than in sheep with long fleeces (14). As a result of the hyperaemia of the skin, the wool growth is affected (14). Shortly after the development of the mouth lesions often a secondary aspiration pneumonia occurs, caused by oesophageal paresis and vomiting (14).

Sometimes the clinical signs include diarrhoea, which may be blood stained.

About 12 days after the onset of the disease a torticollis often occurs.

Congenital defects in the offspring of affected sheep: stillborn lambs; live lambs with spasticity and oedema of the limbs, "dummy" syndrome; hypoplastic brains; cranial cavity largely filled with fluid.

### 8.3 Clinical Signs Arranged in Degree of Specific Relationship to Acute BT (62)

The following listing starts with the most characteristic symptoms:

- oedema
- red or purple lips, tongue or muzzle
- burned or scabby muzzle
- sores or ulcers in mouth
- sloughing of hoof
- swollen tongue
- lame
- swelling of feet
- red feet
- red teats
- peeling of skin
- excessive slobbering
- ulcers on feet
- scabs on teats
- loss of hair or wool

- sudden drop in milk production
- fever
- watering eyes
- red or inflamed eyelids
- dry, flaking skin
- cloudy or ulcerated eyes.

#### 8.4 Symptoms in Local Sheep or Imported Vaccinated Sheep

Short febrile response with passing hyperaemia of the buccal mucosa.

#### 8.5 Symptoms in an Endemic Area

For sheep, see point 4.

In cattle the infection is asymptomatic but viraemia occurs (49).

#### 8.6 Mortality

0-90 per cent depending on virus strain, breed of the sheep, environment, immunity of the animals.

Up to 20 per cent mortality has been reported in endemic areas, but 90 per cent mortality can occur if a virulent strain emerges, which infects susceptible sheep.

Australian data report the main losses among the lambs (this is also a non-endemic area) (3).

During the acute febrile response, death seldom occurs. Mostly, sheep only die 6-10 days after the onset of the disease, because of exhaustion. Probably the most common direct cause of death is bronchopneumonia, often resulting from aspiration of rumen ingesta.

#### 8.7 Prognosis

The course of the disease is hard to predict for individual sheep, because a hopeless looking case may recover, while milder cases may die in the recovery phase. Generally recovery often is very slow and seldom within 10-15 days.

Prognosis of survival for sheep with diarrhoea is poor, particularly when the faeces are bloodstained.

After 3-4 weeks large parts of the fleece fall out in recovering animals.

## 8.8 Rank Order and Frequency of Clinical Signs Registered in an Outbreak among Mississippi Cattle

1979: 13,000 head of cattle included in the survey, morbidity one per cent (62).

- Lameness
- Fever
- Burned or scabby muzzle
- Excessive slobbering
- Red or purple lips, tongue or muzzle
- Sudden drop in milk production
- Watering eyes
- Red feet
- Swelling of feet
- Ulcers on feet
- Dry, flaking skin
- Loss of hair or wool
- Scabs on teats
- Red or inflamed eyes
- Red teats
- Sores or ulcers in mouth
- Peeling of skin
- Cloudy, ulcerated eyes
- Swollen tongue
- Sloughing of hoof.

## 8.9 Comparison of Clinical BT and Foot-and-Mouth Disease in Cattle

Acute clinical symptoms in cattle (South Africa [87]) may be very similar to foot-and-mouth disease (FMD).

- transient febrile response,
- excessive salivation,
- nasal discharge,
- dermatitis: pytiriasis and necrosis of the skin with sloughing and growth of new underlying epithelium,
- localized inflammation with necrosis of the buccal mucosa, foul smelling,
- ulcerative lesions on tongue, nose and muzzle,
- oedema of the lips,
- skin lesions of the udder,
- excoriation of the epidermis in the interdigital space, stiffness of gait, laminitis usually in all four limbs,
- coronitis-to the extent that the horny laminae slough,
- lameness,
- erosion or ulcers on the dental pad,
- loss of condition.

Unlike in sheep, the distribution of the mucosal lesions of BT in cattle CAN NOT BE USED TO DIFFERENTIATE IT FROM FMD.

Peak fevers develop in cattle usually about the same time as the hyperaemia of the mucous membranes and may reach 41°C, but fevers from 39.5-40.5°C are more common. The most frequent lesion is a superficial ulcer on the dental pad.

The clinical symptoms of BT in cattle could be the result of a hypersensitive reaction induced by prior exposure to other serotypes of the BT or other related viruses (62) (see 8.10).

#### 8.10 Allergic Reaction in Cattle? (62)

In non-immune sheep there is no marked age difference in susceptibility. But in cattle the morbidity is higher among the older animals. It has been suggested that clinical BT in cattle is a hypersensitivity reaction and previous exposure to BTV of a different type or other related viruses predispose cattle to development of clinical BT disease. There is no correlation between the age of the affected cattle and the severity of the clinical signs.

The intensification of the clinical respons by previous oral administration has not been explained (60).

#### 8.11 Subacute or Chronic BT Disease in Cattle

The signs shown by affected cattle may be acute, subacute or chronic (14). Clinical symptoms of chronic BT disease (57) are:

- chronic diarrhoea,
- abortion,
- excessive hoof growth.

#### 8.12 Morbidity and Mortality

In many cases, cattle with a viraemia do not show clinical signs of the disease. The morbidity in infected herds in the USA varies greatly, but typically about 5 per cent of the cattle are visibly sick (41). The mortality in cattle is low, usually under 5 per cent. (9).

#### 8.13 Effects on the Genital Tract of Cattle

Bluetongue virus is abortogenic and teratogenic in

pregnant cattle exposed to the virus in the first or early second trimester of gestation (57).

In cattle, fetuses infected between 80-125 days gestation are killed by serotype 11 (and EHDV), whereas serotypes 10, 13 and 17 cause hydranencephaly (76).

The effects in heifers are abortions and congenital anomalies (excessive gingival tissue, dwarfism, arthrogryposis and hydranencephaly).

## 9. ECONOMIC SIGNIFICANCE

Direct losses due to mortality may be high, but the indirect loss may be of even greater economic importance (14).

### 9.1 Loss of Production because of

- loss of condition (due to a.o. muscle degeneration),
- very slow recovery (weeks, months),
- loss of the fleece,
- reduction of milk yield in affected ewes and cows,
- abortion, production of weak or malformed lambs and calves,
- affected animals become more susceptible to secondary infections,
- the breeding efficiency of infected sheep is affected. Barren sheep generally go into anoestrus and may skip at least one breeding season (14).

In cattle, the most significant losses are frequently due to infertility, abortions, deformed and weak calves in chronically infected herds. The acute clinical signs of stomatitis and laminitis are rarely seen in these herds (63).

### 9.2 Export Restrictions

Some examples:

Barbados repeatedly had difficulties in exporting pedigree Barbados black belly sheep because of antibodies to BTB (26, 92).

Water buffalo exportation in Trinidad was delayed when approximately 95 per cent were found to have antibody to BTB.

Germ plasm cannot be traded free. The export of breeding



stock and germ plasm from the USA to Australia and Europe is very difficult because of the prevalence of BT antibodies among cattle and sheep.

## 10. PATHOGENESIS

The first virus replication occurs at the site where the mosquito attacks. After experimental subcutaneous infection the spleen, tonsils and certain lymph nodes are the reproduction sites before the onset of a detectable viraemia. BTV replication also occurs in other lymphoid tissue.

In sheep an increase in blood titer commences up to 36 hs before appearance of clinical signs or lesions (22).

The distribution of the virus in the blood during viraemia is as follows: there was found that washed erythrocytes contain 10-100 times more virus than the buffy coat fraction and  $10^3 - 10^6 \times$  the concentration in the plasma fractions. These values are true for the early, acute and convalescent stages of BT infection in sheep, cattle and goats (41) (see also 4.3).

After the viraemia the virus replicates at the so called predilection sites. The virus has an affinity for the endothelium, periendothelial cells and pericytes of capillaries, precapillary arterioles and venules.

Maximal virus concentrations can be found in the underlying small vessels of the epithelium of oral mucous membranes, skin, coronet of the hoof (14). Also the reticulo-endothelial cells of the lymph nodes draining the tissues of the head contain the virus.

The striated muscles are also mentioned as being a predilection site, as well as the mucous membranes of the gut (49).

Lesions develop in certain tissues subjected to the greatest amount of mechanical stress (14):

- lower lips opposite the incisors,
- dorso - lateral aspects of the tongue opposite prominent molar teeth,
- certain parts of the fore-stomachs (muscular pillars, oesophageal groove),
- pylorus.

The swelling, necrosis and subsequent hyperplasia and hypertrophy of the endothelial cells result in vascular occlusion. The overlying epithelium develops lesions because of lack of oxygen.

There is some suggestion of a correlation between the distribution of lesions and temperature of the host tissue, because the most severe lesions are invariably observed in tissues exposed to the environment.

Also is BT more severe in recently shorn than unshorn sheep, which may be because of sunlight hypersensitivity (see 8.2).

## 11. PATHOLOGY

The most prominent lesions of the digestive system are usually found in and around the mouth. Ulcerations in the nose can be found and hyperaemia of the oesophagus occurs. Hyperaemia of the ruminal papillae, ruminal pillars and reticular folds is commonly observed. Petechial haemorrhages are present in the mucosa of the abomasum and the subserosa, encircling the pylorus, is often diffusely hyperaemic (14).

Distinctive haemorrhages, ranging in size from 2-15 mm are consistently found in the tunica media at the base of the pulmonary artery and are generally considered as pathognomic of BT (14). In the USA the necrosis of the papillary muscle of the left heart ventricle is considered to be one of the most important diagnostic data. To check for this necrosis, it is necessary to make a cross-section of that particular muscle because the necrotic area is localized in its centre (44).

The lymph nodes, particularly those draining tissues of the head, are commonly enlarged, oedematous and haemorrhagic.

The spleen may be slightly enlarged.

Dark red and gelatinous areas may extend from the subcutis into the intermuscular connective tissue all over the carcass. The skeletal muscle lesions consist of petechial or ecchymotic haemorrhages (1-2 mm of diameter). It is important to examine the muscles very closely, in order not to miss these haemorrhages. The best way to look for these haemorrhages is to hold a thin slice of muscle against the light. Hyaline degeneration causes a spotted appearance of the skeletal muscles and is mainly found in the muscles of the thighs, shoulders, back and neck (14).

Degeneration of the heart muscle also occurs, which possible is an explanation for the sudden death of only slightly affected animals (96).

### Pathology of the foetus

The following signs may be found: oedema and haemorrhages in the liver, the adrenal medulla and the heart; a meningitis of brain and spinal cord.

## 12. DIAGNOSIS

### 12.1 Field Diagnosis

- (a) Anamnesis: how has been the development of the disease?
- (b) Epidemiology.
- (c) The area where the disease emerges.
- (d) The season of the year.
- (e) Clinical signs: most important are the overall clinical signs in a herd of cattle or flock of sheep (bad condition, loosened wool, characteristic foot lesions), or in a more acute stage in a sheep flock (fever, oedema of the head and neck and swollen congested mucous membranes with ulcerations).  
In cattle the characteristic low morbidity makes the disease more difficult to diagnose (63).  
Generally, when most animals already show mouth lesions, the slight elevation of body temperature, which may occur is no longer of diagnostic importance.
- (f) Post-mortem: for the field diagnosis, the finding of petechiae in the muscles of the shoulder is important (38).  
In addition, haemorrhages at the base of the pulmonary artery are not to be overlooked.  
In the USA, the necrosis of the papillary muscle of the left heart ventricle is important.

### 12.2 Laboratory Diagnosis

To confirm the diagnosis, the virus has to be isolated and identified, or rising antibody titers from the acute to

the convalescent stage of the infection have to be demonstrated.

Seropositive or seronegative results of a single test for antibody to BTV imply little about the recency of infection or about the current virologic status of the animal (38). Because of these two reasons, it is not possible to use the results from individual animals to indicate virus prevalence (37).

The cross-neutralizing antibodies produced after infection with a given virus type in a group of animals will be variable. This will depend on the responsiveness of individual animals. High frequency of "clusters" found against one or a few types only, may be suggestive for actual infection and that all remaining antibody responses are cross-reactions (37).

The best sample for isolation of BT is fresh whole blood. Blood has to be taken from animals with fever, but still free of typical symptoms. A volume of 10-50 ml blood must be collected in Heparin or other anticoagulant such as sodium citrate. The blood has to be transported to the laboratory on wet ice. If the shipment will last more than 48 hs, the samples must be centrifuged and the washed red blood cells (RBC) can be sent to the laboratory on wet ice.

The washing procedure is as follows:

- remove the plasma after centrifuging,
- resuspend the RBC's and buffy coat in normal saline containing one per cent phenol and recentrifuge,
- discard the supernate and resuspend the RBC's in sufficient phenolized saline to restore the sample to its original volume.

The use of glycerol-oxalate-phenol preservative mixture (OPG = potassium oxalate 5 g, phenol 5 g, glycerine 500 ml, distilled water 500 ml) is an excellent stabilizer for the virus and enhances its isolation (29, 42).

If RBC's are used, quantitation of virus is dependent on ultrasonic disruption of blood samples (29).

After the acute phase of the disease, it is possible to take the spleen or red bone marrow from an animal that has died. To collect bone marrow samples, the entire femure of a calf can usually be shipped to the laboratory on wet ice, or the sternum of a mature animal (63).

## 12.2.1 Laboratory tests for virus identification

### (a) Neutralization test

With a virus neutralization test (VN), serotype specific antibodies can be identified. Cattle, sheep and goats show this type specific neutralizing response.

Paired sera are ideal to identify the infecting type. Sometimes low levels of cross-neutralizing antibody can be found to other than the causing agent, even after first infection. These titers will be low and do not negate the value of the neutralizing antibody diagnostic test. Only in cattle, some difficulties exist in respect of this diagnostic test:

- cattle produce more frequently cross-neutralizing antibody than sheep or goats,
- some individuals may develop antibody levels, that are only of diagnostic value during a month or two after infection.

Repeated exposure of animals to BTV in general increases the level, valency and duration of cross-neutralizing antibody. Ultimately it will be impossible to diagnose the virus types in a certain area by this test. In an unknown area, the best approach for a virus survey is the collection of sera only from young animals (up to 18 months) that have undergone not more than one or two infections (26, 92).

Modification of the test: antibody-clusters titer estimation (26, 92). With this calculation technique, the neutralizing antibody titers of a group of animals are analyzed. Cross-neutralizing antibody titers are estimated to occur at low level and at random. In this way it is possible to differentiate cross-neutralizing antibody titers from infecting virus type(s) antibody titers.

Plaque reduction neutralization test: When L-929 cells are used, BT plaques appear in 48 hs and after four days post-inoculation the numbers are stabilized (6). At present it is the most sensitive serological test for detecting reactors (29).

The calves from dams infected during pregnancy demonstrate SN (serum neutralizing) antibodies in their serum after taking the colostrum. Maternally acquired BTV neutralizing antibody is only detectable for 60 days, which of course is a function of the sensitivity of the test used (40).

Neutralizing antibody is often detectable earlier in the course of infection and for a longer time than are CF antibodies (98). Neutralizing antibodies can be detected 14-30 days after natural infection and will persist more than 12 months, or perhaps for life.

(b) Agar Gel Immunodiffusion Test (AGID)

With the AGID-test it is possible to determine the prevalence of antibody to the common group antigen of BTV (26, 92). These antibodies can usually be detected between 14 and 28 days after infection and persist for about three years (26, 92). Calves of dams infected during pregnancy have AGP antibodies in their sera after taking the colostrum. No AGP-antibodies can be found after the 50th day of life (57). However, others report that group-specific colostrum antibodies in calves disappear within a few weeks until four months (40) (AGP-test).

The AGID-test has been widely used for serological surveys in many parts of the world. A serological survey of 6,274 sera using this test has been described (26, 92). The test (modified Ouchterlony) was also used in a survey in Iran in 1974 (1).

Disadvantages of the AGID-test:

- AGID-test detects cross reactive antibody to orbiviruses other than BTV because of the complex serological inter-relationships of the orbiviruses. Even with this limitation the AGID-test was successfully used in Australia in spite of the existence of many orbiviruses (26, 92).
- The BT immunodiffusion test (BT-ID-test) used in the USA often is negative at the time of virus recovery (76). In an epidemiologic survey in California, 43 per cent of the cattle and 23 per cent of sheep from which BTV was isolated were AGP negative! (74).
- The test does not provide information on the titer of the serum (90).

The BT-ID-test is more reliable, easier and more reproducible than the modified direct complement fixation (MDCF) test (62, 98), but less sensitive than the enzyme-linked immunosorbent assay (ELISA) (26, 92).

The antigen used for the test can be a cell culture-derived antigen (see chromatofocusing) or —when the laboratory is not equipped for cell culture— a BTV-infected newborn mouse brain antigen.

Remark: The mouse brain ID-antigen is highly efficient in detecting BTV antibody activity in ovine sera, but not as good as cell culture-derived antigen for testing bovine sera (32).

The test provides results usually within 18 hs, and not later than 48 hs (32).

IgG is the main antibody reactive in the AGID-test.

Micro agar gel precipitation test (AGP) (6): The results are closely parallel to the MDCF-test when a TC pressure dialysed antigen is used. The fairly large amount of this special prepared antigen required for the test is a disadvantage. The test is not adaptable to the titration of antibodies in a serum. On a limited number of samples, this test can serve as an additional check.

Chromatofocusing (46) and isoelectric focusing (36): The Research Institute of Tubingen, West Germany, developed a technique to isolate a group-specific antigen of BT by means of chromatofocusing. With this technique BTV induced protein (=Protein 7= P7) is purified from the infectious supernatant of cell cultures. In ID-tests, this purified P7 always shows only one clear precipitation line. The P7 non-infectious BT group-antigen can also be obtained by exclusion chromatography and two cycles of iso-electric focusing (36).

This purified antigen can be used as a diagnostic reagent.

(c) ELISA-test

The ELISA-test (47) is more sensitive than the AGID-test (26, 92). The P7 antigen prepared according to the chromatofocusing technique (see AGID-test) is suitable to use in this test. Group-specific antibodies can be detected as efficiently in the ELISA as with the MDCF-test (90).

(d) Modified direct complement fixation test (MDCF)

This test cannot be used for serotyping the BT strains, since it only demonstrates the sero-group reactivity.

The CF antibodies occur 10 days after the start of fever.

The circulating antibody levels remain detectable during 1-3 years after the original test (in endemic areas) (6).

The MDCF-test is less sensitive than the BT-ID-test. The test is valuable for the diagnosis of the disease (98).

The MDCF-test has been accepted as a standard requirement by countries requiring serological testing of cattle in regard to BT (43). The serum for this test is preferably collected from fresh blood, rather than whole blood in OPG (43).

Some disadvantages of the test are:

- sometimes difficult to estimate the serum-titers due to the many variables involved in the test,
- often hard to obtain modifying factor from suitable donor calves,
- sera of some cattle and sheep tend to act anticomplementary (90).

It is mainly IgM, that is reactive in this test.

(e) Haemagglutination inhibition test (HI)

This test is recommended as a new method to identify the various BTV serotypes (45). Serum must be treated to remove nonspecific inhibitors before being used in the HI test. A variety of erythrocytes can be used for this test, including those of sheep, fowl, guinea-pig, mouse.

(f) Fluorescent antibody technique

By this method it is not possible to differentiate between strains of BT-virus (like the MDCF and AGP-test). It is a good test to differentiate BT from epizootic haemorrhagic disease (EHD) (6).

(g) Hemolysis in gel (HIG) serologic test

This test has been developed in the USA and is sensitive and distinguishes readily BT from EHD virus antibodies (75).

(h) Monoclonal antibodies

The use of monoclonal antibodies is important at the moment for diagnostic purposes (Callis, J.J., personal communication, Nov. 1983).



(i) Serological tests used in the USA

Tests for antibodies used since 1969 in the USA are (65): AGP, MDCF and BT-ID-test (62).

(j) Polyacrylamide gel electrophoresis (PAGE)/fingerprinting/genetic drift

Bluetongue virus isolates cannot be identified regarding to serotype by their electrophoresis migration pattern. Within one serotype there is a large variation in some genome segments, revealed by the PAGE (differences in electropherotype).

The genetic variation does not appear to be related to either the geographical location of the isolation, the year the isolate was made, or the species of animal from which the isolate was obtained, suggesting that genetic variation of BTV is constantly manifest (85).

Also oligo nucleotide fingerprints proved that a strain isolated from one and the same area undergoes recombination of RNA segments and genetic drift both (90). These investigations extended over a 12-year period. Also the subtypes collected in different regions but in the same year show differences in the fingerprint picture.

(k) World reference laboratories

- South Africa: World Reference Laboratory for Bluetongue, Onderstepoort.
- United Kingdom: Animal Virus Research Institute, Pirbright.
- USA: Plum Island Animal Disease Center, New York.
- Canada: Animal Diseases Research Institute, Ottawa (70).

12.2.2 Virus Isolation

(a) Carriers

An interesting phenomenon is the very early appearance of neutralizing antibodies in BT-infected sheep and the co-existence of high titers of virus and neutralizing antibodies. This seems to indicate a firm adsorption of the virus to the

erythrocyte membrane or localization within or outside the membrane in a way, which protects the virus from the neutralizing effect of the antibodies (19). It is of great practical significance, that the virus often can be isolated from washed erythrocytes, when whole blood yields negative results (14).

Nothing is known about the sites of BTV replication in latently infected cattle or in what form the virus is present in the animal (58). But the virus is in close association with erythrocytes in the presence of virus specific serum antibody (57). Subinoculation of blood from almost any clinically healthy bovine during the late summer months in South Africa could be relied upon to produce BT in susceptible sheep even though specific virus-neutralizing antibodies were present in the same blood sample (41).

The carrier-time seems to depend on the type of the virus.

In literature a carrier state of 16 weeks in cattle is recorded (41). But in many cattle the virus persists for a much shorter period. One bull, born to an infected dam, was shown to maintain a viraemia for three years, when held in insect-secure quarters (41). Calves born to dams infected in certain stages of gestation may become persistently infected with BTV. This type of viraemia can exist with or without specific BT neutralizing antibodies (41, 58).

Sheep are known to circulate virus in high concentrations and for as long as 31 days (15).

Goat sera collected on the 14th day after experimental infection contained a high level of neutralizing antibodies although virus could still be isolated from the blood collected a few days later. Goats can harbor the virus for even longer periods than sheep (15).

The isolation technique is important to obtain a positive result from a carrier.

(b) Virus isolation from carriers by inoculation of sheep, embryonated eggs or cell culture

It is not easy to conclude which is the most sensitive method to isolate BTV from virus carriers when reading the literature.

Most authors are of the opinion that inoculation of washed erythrocytes in susceptible sheep gives the highest

rate of BTV from suspected BTV-infected domestic ruminants (59). For detecting chronic BT viraemia the washed erythrocytes/sheep method appears more adequate than chicken-embryos-inoculation (42), since BTV presumably primarily is associated with the blood (see also 10. Pathogenesis: distribution of the virus in the blood). For example, when virus could not be demonstrated for more than 28 days using embryonated eggs, it was possible to isolate virus with the washed erythrocytes/sheep method for as long as 100 days! (41).

This greater sensitivity might also be explained by the greater amount of inoculum used in sheep than in eggs and a greater pathogenicity of special strains for sheep than for ECE (57).

In contradiction with the foregoing are the following statements:

- Some authors reported that inoculation of the chorio-allantoic membrane of ECE and incubation temperature of 33,5°C was a more susceptible system than either cell cultures or sheep (59).
- Others found that intravenous inoculated ECE are as sensitive as sheep for the primary isolation of BTV. Nevertheless in an isolation trial only one embryo out of ten inoculated produced detectable virus (33).

Conclusion (43, 62): The combination of egg and sheep systems appears to be the most reliable, because both eggs and sheep failed to demonstrate virus on some occasions. This combination of test systems could well prove to be the preferred method for certificating animals destined for export.

(c) Washed erythrocytes method

The 3-times washed erythrocytes should be used immediately for ECE or sheep inoculation or stored at 4°C. Luedke *et al.* (57) described a modification of this technique by which the buffy coat is not removed because it contains BT only during the acute stage of infection and the concentration of the virus is never as high as in the erythrocyte fraction. Only one washing of the erythrocytes is already effective to remove the plasma.

(d) Blood autograft technique in sheep

When sheep are used as the BTV assay system, washed

erythrocytes are inoculated. In this procedure the blood autograft technique is used in lieu of attempting serial passage in sheep.

On 5, 6, 7 and 8 days post-inoculation (DPI), blood is collected from each inoculated test sheep and 10 ml immediately injected subcutaneously into the same sheep. Body temperatures are recorded on test sheep twice daily until 28 DPI. Following 28 DPI, each test sheep is subjected to the MDCF-test. The mechanism of the blood autograft procedure is not understood.

(e) Egg inoculation

A chorio-allantoic membrane or yolk sac inoculation is done. After 2-4 days the embryo will die with a red appearance because of the hyperaemia and haemorrhagics on the membranes. Often many passages are needed until a regular death of the embryo occurs at day 2-4.

It was shown that 10-11 day old chicken embryos proved to be 100-1000 times more sensitive to BT when inoculated intravenously than 8-day old embryos inoculated in the yolk sac (30). When for example 28 successful primary virus isolations were done for type 16 BT-virus by the intravenous inoculation of ECE, only 70 per cent were positive with yolk sac inoculation.

(f) Tissue culture

Many cell-lines can be used. Often used are lamb kidney monolayer cultures, but they are less readily infected and the cytopathic effect is less extensive (after 1-8 days) than other cell lines (78).

Bluetongue virus also grows in bovine embryo kidney cells. After one or more passages in eggs, BTV grows in BHK 21, VERO and L-929 cell cultures (78). Other cells used for cultivation of the virus are: mouse fibroblast cells (88), fetal bovine bone marrow cells, bovine macrophage cultures, organ cultures of tracheal rings (29), calf testis, C 6/36 (=insect cell line) (28, 54), IBRS-2 (cells in suspension in roller tubes) (82), monkey kidney stable (MS) cell culture, fetal ovine lung cell line (73).

The different BT-strains do not grow at the same rate.

When the virus is multiplying, tubular structures appear in the cell-cytoplasm, which are characteristic of orbiviruses.

(g) Primary isolation of BTV field strains

The primary isolation of BTV field strains in tissue cultures is not satisfactory. The better method is to do one passage of field material in eggs before introducing it into a tissue culture (30).

One flock may often be infected with more than one strain of BTV. More than one serotype of virus has been isolated out of a single animal, although normally cross-neutralizing antibodies exist after the first infection (40, 89). Also experimentally it has been possible to demonstrate simultaneous circulation of more than one strain of BTV in animals infected by the intravenous inoculation of polyvalent vaccine (31).

The isolation and typing of BT-virus strains can be achieved within 10 days (30).

(h) Virus isolation from semen

During viraemia, BTV can also be found in bull semen. Semen can be assayed by intravenous inoculation of ECE, or by subcutaneous injection into sheep (72).

12.2.3 Virus Antigen Production

To obtain virus antigen, baby mice are infected intracerebrally with egg-adapted strains. Field material is not suitable for this purpose (44).

Suckling mice of 1-4 days old are susceptible when inoculated intracerebrally. Older mice produce smaller amounts of virus (see also AGID-test: Chromato Focusing).

13. DIFFERENTIAL DIAGNOSIS

- Foot-and-mouth disease: BT and FMD may be difficult to differentiate from each other. In sheep some old FMD stages look like BT. BT is a "necrotic" disease and FMD a vesicular disease, but after the vesicles have erupted, the appearance of the mucosa can be the same in these two diseases.

The distribution of the mucosal lesions of BT in cattle is not diagnostic. In sheep the lenticular necrotic ulcers, which sometimes develop on the lateral aspects of the tongue, are pathognomonic for BT (see 8.2).

Because the clinical signs may not be very different between FMD and BT, the final diagnosis/differentiation can only be done by laboratory investigation (see 8.9).

- Vesicular Stomatitis (VS) (see FMD).

- Rinderpest: The mouth lesions can resemble BT. Differences are: the incubation period and duration of the disease in rinderpest are shorter, rinderpest can occur during the whole year, and in contrast with BT, rinderpest is easy to transmit from sheep to cattle.

- Contagious Pustular Dermatitis (see Contagious Ecthyma).

#### Two diseases with a rapid T-rise

- Heartwater (sometimes existing at the same time as BT). In heartwater the febrile period is shorter, there are nervous symptoms, the brain smear is positive, and heartwater is responsive on tetracyclines.

- Arthropod-borne Encephalitiden (Rift Valley fever and Wessels-Bron virus): No focal necrosis or acidophil inclusion bodies in the liver. In case of doubt, laboratory confirmation will be needed.

#### Diseases easy to confuse with ulcerative stomatitis or secondary infections

- Sheep Pox: The disease does not cause foot lesions, and the pocks are not restricted to only the mouth.

- Contagious Ecthyma: Papules and pustules in this disease, instead of hyperaemia, oedema and ulceration as in BT (see Contagious Pustular Dermatitis).

#### Cattle diseases

- Sweating Sickness: Has the same seasonal occurrence as BT. Differences: affects particularly young cattle, occurs only in the warmer regions, there are large excoriations of the epithelium of the skin, in contrast with BT, and is not transmissible by blood.

- Three-day Stiff Sickness: Stiffness and paralysis are more prominent than in BT. There is no hyperaemia or necrosis of the mucosa of the mouth; does not occur in sheep under

normal circumstances, and is not transmissible to BT susceptible sheep.

- Diphtheria in calves: Gives well defined, more severe lesions in mouth and on the larynx, is restricted to calves, and is not transmissible by blood.

- Bovine Viral Diarrhea (BVD).

- Infectious Bovine Rhinotracheitis (IBR).

- Malignant Catarrhal Fever.

- Mucosal Disease.

- Mycotic Stomatitis.

- Photosensitization (41).

- and of course, FMD and VS.

Pay special attention to the season!

#### Other Diseases

- Stiff Lamb Disease (lack of vitamin E).

- Disturbance of the blood circulation in the hoof by reason of metabolism failures.

- Ibaraki Disease: Causes a severe BT-like disease in cattle but does not affect sheep (99). It has only been seen in Japan.

- Epizootic Haemorrhagic Disease of Deer: The disease is clinically identical in cattle and deer. Present in Canada, the USA (19, 78), large part of the Caribbean area (37), Australia and West Africa.

- Pneumonia: Whenever high mortality occurs due to pneumonia, in late summer or early fall, BT should be considered.

#### 14. THERAPY

The therapy consists of symptomatic treatment only. Because of the mouth lesions it is recommended to feed only soft green food. Mouth and nose can be repeatedly sprayed with a

solution of low concentration of a non-irritating disinfectant, as K-permanganate, or fluids with alum and glycerine.

It is very important to check regularly the motility of the rumen of recovering animals. A good quality food in this phase is of great importance.

Infected animals have to be kept out of the sun, because the sunlight deteriorates the symptoms when skin lesions exist. Superficial skin lesions can be treated with solutions to prevent secondary infections.

Small quantities of laxative are recommended, for example sugar.

## 15. CONTROL AND PREVENTION

### 15.1 Import Rules for Live Animals and Animal Products

Due to the fact that it is virtually impossible to eradicate infection once it has become established, countries free of the disease should take every possible precaution to prevent introduction of the disease through the entry of cloven-hooved animals or insect vectors (14).

Formerly, in the USA all ruminant livestock including those for zoological collections—other than from Mexico or countries considered free of infection—were examined for evidence of infection with BTV before importation (26, 92).

A recent decision by the United States Department of Agriculture (USDA) to allow importation of ruminants with serological evidence of prior infection with unknown types of BTV has caused considerable concern among producers of sheep and cattle, and wildlife conservationists. The decision was made despite the fact that only five of the 23 types of BTV have been isolated in the USA, and the acknowledged insensitivity of existing diagnostic techniques for certifying livestock free from infection. The decision has been opposed by the United States Animal Health Association (25).

The former regulations of the USA regarding the importation of ruminants with relation to BTV were as follows (25, 75): BT-ID tested seropositive animals were identified and blood samples were submitted for virus isolation. If BTV was isolated from any animal out of the group, the whole group was denied importation into the USA. If no BTV was isolated from any of



the animals, the whole group would be permitted entry as far as the BT status was concerned (25, 75). It should be noted that there always is the risk to import carriers, which are serologically negative (see 4.4). This became very clear when, in February 1980, 60 Zebu-cattle from Brazil were admitted to the Harry S. Truman Animal Importation Center, in Florida, USA (Fleming Key, Key West, Fla.). These cattle were extensively tested for many diseases during their quarantine period in Brazil and positive animals were disqualified. However, at arrival at the Quarantine Center in Florida, four animals were shown to have developed BTV antibodies, during the 30 days since their last test in Brazil. During their 150 days quarantine period in the USA, four more animals developed BTV antibodies. From one of the eight positive reactors in the serologic tests virus was isolated. This was BTV type 4, exotic to the USA (25, 33).

The official test for export to Canada from the USA is the BT-ID test. See also the American literature concerning this test (JOCHIM, M.M. Improvements in the AGP-test for BT. Proc. Ann. Meet. Am. Assoc. Vet. Lab. Diag. 19: 361-376, 1976; PEARSON, J.E., JOCHIM, M.M. Protocol for the immunodiffusion test for Bluetongue. Proc. Ann. Meet. Am. Assoc. Vet. Lab. Diag. 22: 463-471, 1980).

There is no justification for restricting the movement of animals within the Caribbean area (26, 92).

Virus may also be carried in semen and ova and the import of such materials from infected areas should be avoided (78) (although it is noted in the literature that embryo transfer is not associated with transmission of BTV [75]).

A procedure for certifying that bulls with BTV antibodies are free from active virus infection is described in the USA (69). The protocol includes inoculation of blood from the bulls into embryonated chicken eggs and susceptible sheep, as well as inoculation of semen into sheep.

For import into Australia, semen is stored in quarantine for twelve months before entering the country. This period allows any serious disease to declare itself in the domestic livestock of the exporting country. Beyond this, the donor bulls have to be MDCF negative on the first day of collection of semen and every 60 days thereafter during the collection period, with a final test 30 days after the last collection (43). Imports from countries where the disease is endemic are impossible. Airplanes from these countries have to be sprayed inside with insecticide after take-off. Imports preferably have

to be made during the winter months and the animals have to be checked on the presence of virus during more than 30 days. It is better to avoid importation of pregnant animals (40). Meat, wool and leather do not transmit the disease and can be imported without any restriction. However, after BTV was discovered in Australia, the country encountered numerous problems with the export of the above-mentioned products (100).

## 15.2 Eradication

After BT emerges, attempts must be made to minimize the losses as far as possible (16). Spain and Portugal are the only countries where BTV was successfully eradicated. The outbreak began in 1956 in Portugal and spread to Spain within a month. The epizootic was very severe with a mortality of 75 per cent in affected sheep flocks. The measures taken to eradicate the disease were: quarantine, slaughtering the infected sheep, and compulsory annual vaccination of all sheep. The last outbreak occurred in 1960. The vaccination ceased and the clinical disease has never recurred in either country (22).

Remark: The *Culicoides* vector was not eradicated in this campaign!

Eradication measures depend on the economic situation of the country and the extent of the area where the virus is active.

In Australia the eradication plans are as follows: slaughter of all the ruminants in the infected area, disinsection of the infected area, and maintenance of a larger quarantine zone in which the standstill of ruminants is enforced.

An infected area is defined as the area within an approximate five-mile radius of known infected herds.

A quarantine zone can be extended about 50 miles beyond the infected area, depending on prevailing wind patterns, topography, location of livestock, etc. These measures have to continue up to eight months after the last clinical case of the disease (22).

In reality, where serologically positive cows were found, the government only declared standstill of cattle in certain areas during three months. This was estimated to be sufficient because of the absence of clinical cases (52).

### 15.3 Control in Endemic Areas

In endemic areas, little can be done except to refrain from importing exotic breeds of sheep or to vaccinate those that are to be introduced (81).

### 15.4 Vaccination

Vaccination is the main method of prevention.

Live attenuated vaccine: The modified live-virus vaccine is empirically attenuated by serial passages in eggs and produced in tissue culture systems (16). The serial passages in egg embryos rapidly reduces the virulence of the virus (29).

Monovalent vaccine gives a good protection. The administration of a single potent strain is followed by a durable life-long immunity (16). With the polyvalent vaccines interference can occur (78), and it appears difficult to obtain effective polyvalent immunity by vaccination of a polyvalent vaccine (16).

Problems in Africa with the polyvalent vaccine are (44): interference between the strains, difference in immunizing capacity, growth velocity differences between the modified strains, and different immune response of individual animals to the vaccine.

In South Africa, where at least 17 strains exist, the live vaccine is given as three injections with three different strains (78). Vaccination has to be repeated yearly (16). Workers in South Africa also have experimented with pentavalent vaccine which they apply at intervals of at least three weeks. Sheep, vaccinated according to this regimen, develop antibodies to an average of at least eight of the serotypes (17).

Others have reported on the use of a polyvalent vaccine, that includes the 16 principal serotypes, giving a protection of about one year (38).

Experiments with a vaccine produced in monkey kidney stable (MS) cell cultures, containing serotypes 1-16 resulted only in positive titers for 3-8 of the serotypes (2).

Since there are five types active each year in the USA, a vaccine will be required which includes all five BTV serotypes, currently active (88). However, the USA vaccine is a modified live serotype 10 BTV vaccine, produced in fetal bovine

cells and is only licensed for use in sheep. The vaccine was not approved for cattle (98).

In Spain and Portugal a vaccine with four types (egg-attenuated strains) against the fifth heterologic strain is used successfully (44).

In experiments, the administration of two BTV types gave protection against the development of viraemia following the inoculation of a third BTV type; for example, type 4 inoculation only resulted in homologous neutralizing antibody. But the following type 3 inoculation caused a broad heterotypic response. However, in an experiment it was shown that the serial administration of two or more BTV induced heterologous antibodies, but simultaneous inoculation of the same virus types failed to do so (51). It is questioned if it is possible to produce vaccine cocktails consisting of a small number of BTV types, but giving broad heterotypic cover.

The prevention of viraemia when a third BTV type is inoculated could be explained by the development of memory cells, which on stimulation rapidly produce antibody, followed by the abrogation of the viraemia (50).

The functional importance of the humoral and cellular immune response in protection and recovery have to be studied before a modified vaccine can be composed from a few BTV-types, which will give broad heterotypic protection.

Work is still continuing on inactivated vaccines (35, 71).

Fingerprinting of BTV revealed that antigenic drift occurs with high frequency (75). The antigenic determinants are possibly carried by one protein, for which only one of the segments provides the code (31).

Research work is done on the development of a potent inactivated BT-vaccine, to use in pregnant ewes, young lambs which may still possess residual passive immunity, and in non-zootic countries.

Inoculation of inactivated BTV appears to induce some protection to a subsequent challenge test, rather than causing a sensitized condition that led to clinical disease (see also 8.10). However, after two inoculations with inactivated BTV, associated with immuno-modulatory drugs, the disease provoked by challenge closely resembles clinical BT in cattle. In this experiment the control animals, which only received the challenge

dose, did not show clinical signs (87). Some workers reported that the monovalent inactivated vaccine gives an immunity in sheep and cattle for one year or even for life, but the animal may be sensitized and have a more severe response if infected with virus of another type (63).

Inactivated vaccine is being developed, which in sheep induces the production of precipitating, CF-antibody and a positive lymphocyte stimulation but not neutralizing antibody. This lymphocyte stimulation reaction and at the same time the lack of neutralizing antibody can differentiate vaccinated from non-vaccinated sheep after natural infection (98).

Analysis of the relative immunogenic importance of BT viral proteins must await virus purification (50). The immunity after natural infection appears to be at least one year (against the homologous strain) (38).

The development of a cattle vaccine is important in view of the role played by cattle in the BT-epizootiology (16). Although the economic damage of the disease in cattle is insignificant compared to that in sheep, it is possible even more vital in an eradication program to prevent the spread of infection in cattle than it is in sheep (22). The creation of an immune cattle population would be of great significance in the control of the disease (16).

#### Vaccination scheme for an endemic area

Yearly vaccination of all the sheep with a modified live polyvalent vaccine, at least one month before the begin of the rainy season. Non-pregnant ewes have to be vaccinated at least three weeks before the mating season starts, because the vaccination can influence the oestrus. Pregnant ewes cannot be vaccinated in the beginning of the pregnancy, because of the teratogenic effect of the vaccine. Administration of live attenuated BT vaccine to ewes in the early stage of pregnancy (5-10 weeks) may lead to foetal death with resorption, and development of congenital abnormalities such as encephalopathy and deformities of the skeletal system (16).

In trials, the revaccination of sheep during the sixth week of pregnancy did not cause damage to the foetuses (84). After first vaccination male sheep can become temporary sterile.

Animals which will be vaccinated for the first time have to be vaccinated after shearing, because if reactions occur

after vaccination (which are indeed not usual), the wool may be damaged.

The lambs maternal antibodies can neutralize the vaccine virus up to six months. Newborn animals are susceptible to natural infection, although usually infection does not occur until maternally derived virus neutralizing and precipitating antibodies have vanished completely. Then, precipitating antibodies (AGID) and high levels of virus neutralizing antibody against a single BTV-type can be found after a variable period, normally 1-2 months. In an endemic area this does not exceed six months (40).

### 15.5 Insect Control

Eliminating breeding sites or spraying adult midges only interrupt the cycle for 1-2 weeks. Spraying or dipping the animals may be more effective.

It may be noted that no measures against the insects were taken in the eradication campaign of Portugal and Spain.

Biological control appears to be an interesting approach.

The oral susceptibility of *C. variipennis* female gnats to infection of BTV is known to be genetically controlled. In addition, different field populations of *C. variipennis* appear to have varying susceptibilities to different BTV serotypes. If a colony of *Culicoides* could be developed to be resistant to oral infection with BTV serotypes, then the release of BTV genetically resistant gnats could be used to disrupt the BTV transmission cycle without disrupting the ecology of an area (98).

### 15.6 Prevention Measures by Management

- Transfer of the flock to high and dry areas, where no *Culicoides* exists. The height above sea level is not important but the local height of the meadow in relation to the valley is.

- Locking up the sheep in a stable at night (without light in the stable!).

- Smoking fires at night will help to keep the *Culicoides* away.

- Sheep can be dipped for vector elimination.

- Repellents can be applied, specially in short-wool breeds.

- Sheep in enzootic regions should be shorn in early summer to allow some wool growth before the onset of the BT-season in late summer. Recently shorn sheep become infected more readily and usually develop a more severe form of the disease than sheep having a fair wool covering (16).

- A control measure, that seems to be very effective, at least in South Africa, is to run cattle in close proximity to sheep, because the vector *Culicoides imicola* prefers cattle as host (16).

- Autumn lambing is preferred. Spring born lambs lose their immunogenicity about autumn-time, during the peak-months for BT infection. Immunization is difficult because of the persistent maternal antibodies (16).

- Sentinel sheep flocks should be kept at strategic points in a BT-disease free country, to detect subclinical spread among cattle before affecting large areas, without discovering the disease.

#### 16. RECOMMENDED FURTHER INVESTIGATIONS/REQUIRED INFORMATION ON THE DISEASE

- Sites of overwintering of the virus.
- The role of animals other than ruminants.
- Potential vectors.
- Pathogenesis.
- Virus distribution in tissues of infected animals.
- Immunity.
- World distribution.
- Determining the role of latently infected cattle in the epizootiology of BT.
- Development of a cattle vaccine.
- Further development of an inactivated polyvalent vaccine.

- It would be of great advantage to have a rapid, simple and reliable screening test for viraemia, that could be applied to large numbers of cattle blood specimens. The traditional serological test does not detect antibodies until 3-6 weeks after infection. In this time the disease can spread asymptotically (22).

- International standardization of serotyping techniques.

- Establishment of a new pathogenicity index system for BTV isolates, based on biochemical and genetical characteristics.

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## 18. ABBREVIATIONS

AGID	Agar gel immunodiffusion test.
AGP	Micro agar-gel precipitation test.
AGPT	Agar gel precipitation test.
BT	Bluetongue.
BTV	Bluetongue virus.
BVD	Bovine viral diarrhoea.
CFT	Complement fixation test.
DPI	Days post-inoculation.
ECE	Embryonated chicken eggs.
EEC	European Economic Community.
EHD	Epizootic haemorrhagic disease.
EHDV	Epizootic haemorrhagic disease virus.
ELISA	Enzyme-linked immunosorbent assay.
FMD	Foot-and-mouth disease.
HI	Haemagglutination inhibition test.
HIG	Hemolysis in gel.
IBR	Infectious bovine rhinotracheitis.
ID	Immunodiffusion.
MDCF	Modified direct complement fixation.
MS	Monkey kidney stable cell culture.
OPG	Potassium oxalate, phenol, glycerine.
RBC	Red blood cell.
RNA	Ribonucleic acid.
SN	Serum neutralizing.
USDA	United States Department of Agriculture.
VN	Virus neutralization.
VS	Vesicular stomatitis.

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