Bluetongue: Diagnosis and Significance in the Bovine Animal

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Introduction

Bluetongue (BT) is an important virus infection of cattle even though it is clinically expressed in only 5 to 10% of the infected cattle. There are a few bovine practitioners in areas of low BT prevalence who believe this disease is essentially exotic in the United States (US). Other practitioners have had personal experience with the disease and believe it is a "sleeping giant" that causes severe reproductive problems in their clients' herds. Thus, discussion of the disease evokes a wide spectrum of views and attitudes, possibly more than any other infectious disease of cattle. The infection has been referred to as "a political disease,"[1] "a pain in the neck,"[2] "a mystery,"[3] "a riddle"[4] and recently, "a bigger threat than you think."[5] In the World Animal Review article[1] a preceeding editorial comment stated that "bluetongue is one of the diseases that, for many countries, ranks high in the list of exotic diseases that need to be controlled, and that prompted the convening of the FAO Expert Consultation on Emergency Disease Control." Most bovine practitioners' experience with BT is related to serology and the certification of cattle free of the infection. Some practitioners have had direct experience with the devastating consequences of infection that include "abortion storms," infertility stillborns, and various fetal anomalies and dysfunctions. In this report, the authors will attempt to dispel some of the confusion about BT by discussing diagnostics, the perpetuation of BT virus (BTV) and the health problems that infection produces in cattle.

Diagnosis

A. *In the field.*

BT is an infectious viral disease that is transmitted primarily by biting insects. It affects all domestic and wild ruminants and is characterized by inflammation and congestion of the mucous membranes that can lead to cyanosis, edema, hemorrhages, and ulceration of mucous membranes and lameness. In South Africa where BT was first recognized around 1889 in imported European sheep, BT became known in cattle as pseudo foot-and-mouth disease.[6] In view of the fact that BT in cattle is usually inapparent or subclinical, the bovine practitioner must be alert to the fewer than 10% of the cattle that may show the varying clinical features of the infection. Furthermore, he or she must check for signs of infection in other susceptible ruminants in the area. In frank BT, the fever and leukopenia that commonly develop go undetected, and the astute practitioner who suspects BT in a herd of cattle will examine apparently healthy animals in the herd for fever. The first visible sign may be excessive salivation. Inspection of these animals often will reveal hyperemia of the mucous membranes and exposed epithelium, e.g. on the udder and teats. If these lesions become more intense, inflammation and eventual ulceration of the gingival or buccal mucosa, or the tongue may occur. Shallow ulcers are fairly common on the dental pad. In more severe cases, necrosis of the epithelium of the muzzle produces a "burnt muzzle" appearance. However, this severe lesion has only been observed in naturally infected cattle under field conditions. Concurrently with the oral lesions cattle commonly develop laminitis, characterized by hyperemia and swelling of the sensitive lamina of the corium. In severe cases, necrosis in the sensitive lamina can occur and some infected cattle have been known to slough a hoof. A ballooning degeneration in all levels of stratified squamous epithelium occurs and depending on the severity of the infection, pityriasis, hardening, cracking, and necrosis of the skin with sloughing and growth of new underlying epithelium are commonly observed. Lactating cattle frequently develop scabs on the ends of the teats due to irritation from nursing calves or milking machines and these lesions are difficult to manage and heal.

One important consequence of BT infection in cattle is reproductive failure and it will be discussed later in detail. However, in a field diagnosis of bovine reproductive failures, BT can no longer be ignored and must be included in any differential diagnosis.

B. *In the laboratory.*

Laboratory confirmation is required for a diagnosis of BT. This can be achieved by isolation of the virus or by testing for rising BTV antibody titers. In this regard the bovine practitioner may become discouraged for 3 reasons. First, as a matter of convenience because the closest state diagnostic laboratory may not be equipped to do any of the

BT testing. Second, they probably will not become involved in a BT problem until late in the infection cycle when the viremia of cattle is markedly reduced and BTV is more difficult to isolate. Third, they may be uncomfortable with the epizootiology of BT and the variability of BTV serological responses in cattle.

Sheep, embryonating chicken eggs, and cell cultures are the 3 viral assay systems used for isolation of BTV. Unfortunately, BTV isolation can be time consuming and expensive, especially when the virus orginates from chronically infected cattle. Compounding this problem is the fact that there currently are 5 serotypes of BTV present in the U.S.: 2, 10, 11, 13, and 17. All 5 serotypes are known to infect cattle. [7,8] The full potential of each BTV serotype to cause disease problems in cattle is not known. In California, serotype 11 is most frequently isolated but there is no evidence of reproductive disease.[9] However, in the authors' experience, serotype 11 is the serotype most commonly associated with BT reproductive problems in cattle herds. Multiple serotype infections have been encountered in individual cattle[10,11], but usually one serotype will dominate during an outbreak. If individuals in a particular herd of cattle are sensitized to one BTV serotype, then clinical BT disease is more likely to develop upon infection with a second BTV serotype. Sensitization was suspected for a number of years but only recently demonstrated experimentally with the same virus.[12]

The viremia of infected cattle is associated with cells rather than the plasma.[13] The BTV often coexists in infected animals with its specific neutralizing antibodies. The cellular components of a blood sample must be washed to facilitate isolation of BTV from the blood. Since BTV is cell associated, other body tissues rich in blood cells such as bone marrow, spleen, liver, and lymph nodes are more likely to yield BTV for diagnostic purposes.

The diagnosis of BT by testing for antibody in the serum of infected or recovered cattle is highly variable. This variability depends on the age and immunological experience of a bovine when it is exposed to the virus. For example, was the animal immunologically competent as an adult or as a calf or was it immunologically incompetent at the time of exposure. The latter implies an in utero infection or transplacental transmission of BTV and is greatly influenced by the stage of gestation as a bovine fetus begins to recognize BTV at about 150 days of gestation,[14] or as early as 125 days.[15] Serologic responses vary with fetal age and gestational time of infection. The fetal response may be further complicated by the BTV serotype of infection or infection with epizootic hemorrhagic disease (EHD) a related virus; 2 serotypes are present in the U.S.[16,17] Under natural field conditions other factors such as other diseases and physical stress may further influence these responses.

There are two groups of serologic tests that are used: one that detects BTV group antigens, and one that detects BTV serotype specific antigens. The group tests are the more useful to the bovine practitioner and include 1) BT immunodiffusion (BTID),[18] 2) complement-fixation (CF),[19] 3) enzyme-linked immunosorbent assay (ELISA),[20] and 4) hemolysis in gel (HIG).[21] The first BT immunodiffusion type test was called a micro-agar gel precipitation (AGP)[22] test, or an agar gel immunodiffusion (AGID or AGD) test. The BTID is now the most commonly used BT serological test and the antibodies that it detects in an adult bovine (immunologically competent and mature at the time of infection) can last for years. The official CF test is a modified direct CF test (MDCF)[19] and it is still used extensively by many countries of the world to select cattle for importation. However, this test has lost favor in the U.S. because of the variability of test results among laboratories,[23] anticomplementary reactions, and the short-lived but titerable antibodies. The HIG and ELISA are two of the newer tests that have good potential and are rapidly becoming more useful: the HIG test because it is quantitative and will differentiate between BTV and EHDV antibodies, and the ELISA because of its potential for increased sensitivity and quantitation.

The serotype-specific antibody tests are the serum neutralization (SN) or plaque neutralization (PN) tests, which some scientists may also refer to as virus neutralization (VN). These tests are recognized for their antibody specificity to the infecting strain of virus and are more useful in research than for diagnostic work. An important modification of the PN test currently in use is called the plaque inhibition (PI) test or disc method.[24] In this test, filter paper discs are saturated with undiluted serums that contain specific antibodies against the BTV and EHDV serotypes. The test effectively combines a direct and indirect method for BT diagnostic work. The test not only gives valuable information by telling which virus (BTV or EHDV) was isolated, but it also tells which serotype of the virus was responsible for the infection. A shortcoming of all serotype specific neutralization tests is that, if and when another new serotype of virus emerges, the test used alone might give a false negative test result. In summary, for BTV serological testing, there are a battery of tests available to evaluate the immunological responses of infected cattle. Each test has certain advantages and disadvantages and the tests should be used in combinations rather than singly.

C. *Differential diagnosis.*

In the absence of BTV isolations, BT in cattle is underdiagnosed as the rule rather than the exception. Since signs of BT on occasion can resemble foot-and-mouth disease, this disease and other vesicular diseases must be included in the differential diagnosis. Other diseases that should be included for differentiation include bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), malignant catarrhal fever (MCF), photosensitization, rinderpest, and the miscellaneous causes of bovine stomatitis.

Significance in the Bovine Animal

Significance according to Webster's New Dictionary of Synonyms implies a quality or character in a thing which ought to mark it as of importance, but which may or may not be recognized. Much has been written about BT in the world during the past several years and, as was mentioned in the introduction of this article, BT has elicited a variety of views and attitudes. Irrespective of these differing opinions, the significance of BT resides in the character of the disease itself and in its complex epidemiological cycle. In the final analysis, if and when BT is properly diagnosed and evaluated its true significance in cattle should emerge.

A. *Vector-borne.*

By definition BT is an infectious, viral disease of ruminants that is transmitted by insects. Arthropod or vector-borne animal diseases are probably the most poorly understood of diseases by practitioners and scientists who specialize in infectious diseases. Vector-borne diseases involve an interaction of 3 living factors; a vector, a host animal, and an etiological agent. The primary vector of BTV in the U.S. is *Culicoides variipennis.*[1 25 26]

These vectors are more commonly known as punkies, no-see-ums, or as biting gnats. This biting gnat is about ⅛ inch long and only the female takes a blood meal. The female gnat can become infected when it feeds on an animal that has BTV circulating in its bloodstream. The BTV must complete a 10-to 14-day replicative cycle in the gnat where it eventually multiplies in the salivary gland. Once BTV is present in the salivary gland, the gnat can transmit BTV whenever it bites another susceptible ruminant. Once infected, the gnat can remain infected throughout its life and continue as a potential transmitter of BTV. A single gnat can produce 100,000 to 1,000,000 BTV particles. In some geographic areas, populations of the gnat appear to be genetically resistant to infection with BTV. This gnat is found throughout the U.S. and can be found in southern Canada and in northern Mexico. Another possible vector species, *C. insignis,* is found in the southern half of Florida and in Puerto Rico.

The female gnat requires a blood meal before it lays eggs that hatch into larvae. These larvae develop in soft, silty mud with a high content of organic matter. The typical larval breeding sites are found on farms and ranches in muddy areas that are polluted by livestock or human wastes. These areas include the shallow muddy water along the edges of ponds, ditches, and streams. Frequently a larval breeding area is created by the overflow from septic systems and stock water tanks. The larvae change to pupae from which the adult fly emerges. The female gnat is ready to feed shortly after its emergence.

The gnat represents half of the infection cycle and cannot be dismissed as an insignificant animal pest. Furthermore, they do not respect fences or other man made boundaries and readily attack all mammalian livestock species.

Two other types of biting arthropods have been incriminated in the transmission of BT. They are cattle louse, *Haematopinus eurysternus,*[27] and a soft tick, *Ornithodorus coriaceus.*[28] These 2 pests are not capable of flying. The soft tick is found primarily in California and Oregon. Nevertheless, even though their potential as vectors is not fully known, if they are present they should not be ignored as they may be important in the BTV transmission cycle.

Mechanical transmission of BTV is always a possibility because of the high titering viremia. Any procedure that transfers small quantities of blood from an infected bovine, especially during its peak viremia, can result in the transmission of the virus to a susceptible bovine. For this reason, a hypodermic needle should never be used on more than one animal. Also, any flying biting insect can act as a "flying syringe" when virus contaminates its mouth parts. Biting insects should be controlled as much as possible during the vector season.

B. *Virus Serotypes.*

The probability of bovine exposure to BTV is increased because of the plurality of BTV serotypes, the viral host range that includes all ruminant animals, and the broad geographic distribution of the virus. The more serotypes of BTV that are available to the cattle population, with their subtle differing properties of virulence, transmissibility, and ability to establish persistent infections, the more likely individual cattle may become exposed to the virus. As mentioned previously, BTV serotype 11 appears to cause persistent infections in cattle even though other serotypes may predominate during outbreaks, especially BTV serotype 17.[7 29 30]

The wide host range of the virus also greatly increases the chances for viral maintenance in a geographic area.

The distribution of BT as determined by BTV isolation in the U.S. or by serological evidence does not tell the complete story. For example, the virus has been isolated from sheep in 18 states, from cattle in 23 states, and from deer and other wild ruminants or exotic ruminants in zoos in 14 states for a total of 32 states.[31] Most isolations were from sheep located west of the Mississippi River where sheep are more commonly found. They tend to serve as sentinel or indicator hosts for the virus. However, BTV has been known to exist in the U.S. east of the Mississippi River for more than 15 years but is commonly misdiagnosed in cattle as "mycotic stomatitis."[32] "Hemorrhagic disease" (HD) of white-tailed deer caused by either BTV or EHDV has been documented in the southeastern U.S. (10 states) by the Southeastern Cooperative Wildlife Disease Study group since 1971.[33] "HD" is well established in these states and during the last decade has been considered the most serious infectious disease affecting the nation's number one big game animal.[33] HD has occurred seasonally (summer--fall) every year since 1971, but the outbreaks each year are scattered and unpredictable.[34] Since the vector *C. variipennis* feeds equally well

on both deer and cattle there can be little doubt that wherever these 2 species of animals intermingle, transmission of either EHDV or BTV can occur between them. **A valid question is often asked about which animal becomes infected first in the spring of each year. The answer is unknown, but the authors believe cattle are more likely to infect deer because of their greater numbers and proven reservoir potential.**

Serologic surveys of BT in the U.S. also do not tell the entire story even though serologic evidence of BTV infection has been found in ruminants in every state except Alaska and Rhode Island.[31] A national survey was conducted from November 28, 1977 to February 20, 1978 with serum samples from cattle that were systematically collected at the brucellosis laboratories.[31] The prevalence of BTV antibody in adult slaughter cattle (BTID test results) ranged from 0 to 79% with a national prevalence of 18.2%. The prevalence was generally low in the northern states (especially northeast) and high in the southwestern states. The highest prevalence rates were in Puerto Rico and the Virgin Islands where one might expect increased vector activity.

Survey data are valuable, but reflect activity at a particular moment in time and do not accurately reflect the constantly changing status of a vector-borne disease such as BT. A good example of this changing status was demonstrated in Mississippi following a major outbreak in 1979.[35] The national survey of slaughter cattle in the U.S. during the winter of 1977-78 had disclosed 19 (6.8%) of 281 serum samples from Mississippi slaughter cattle to be seropostive to BTV by the BTID group antibody test.[31] The later survey of slaughter cattle in Mississippi after the outbreak and during the winter of 1979-80, using the same protocol that was used previously in the national survey, disclosed 96 (33%) of 293 serum samples to be seropositive for BTV antibody by the BTID antibody test. These results were considered significantly higher than those previously recorded in the state. Since 1978, many bovine practitioners have also become acutely aware of this changing status by being initially located in an area of low BTV antibody prevalence and subsequently experiencing high seroconversion rates to BTV in a number of cattle herds in their area.[29 30 35 36 37]

The competence of the biting gnat as a vector plays an important role in BTV distribution in the U.S. Adult *C. variipennis* populations from the northeastern states were resistant to oral infection with BTV, while populations from western states were susceptible.[1] This information is in conformance with data of the national survey for BTV antibody presence in slaughter cattle.[31] For example, adult populations from New York and Virginia were only about 3% and 1% susceptible, respectively, and the slaughter cattle showed a 0.3% and 4.5% prevalence of BTV antibody.[31] In contrast, adult populations from southern California and New Mexico were about 26% and 67% susceptible, respectively, and slaughter cattle showed 48.2% and 53.2% prevalence of BTV antibody.[31] Later studies though showed that an adult resistant population from New York yielded BTV susceptible offspring upon colonization in the laboratory. These data suggested that an environmental factor might be responsible for these differences in oral susceptibility of adult populations of the biting gnat. This environmental factor or factors responsible for differences in oral susceptibility are expected to be useful in the development of future BT control procedures.

C. *Reservoir Cattle.*

The authors have devoted considerable research effort toward a better understanding of the role of cattle in the BTV epidemiologic cycle. Evidence suggests that cattle are the primary reservoir of BTV. This reservoir characteristic is the single most important factor in the perpetuation of BTV in a geographic area. It is multifactorial in nature and encompasses virus carrier animals, persistent infections, immunologically nonresponsive animals, reproductive consequences, virus in semen, and "overwintering" of the virus. The first evidence that cattle might play a role in the perpetuation of BTV was presented in 1962 in South Africa.[38] The first evidence for cattle involvment in the U.S. was the isolation of BTV from infected cattle in Oregon in 1959;[39] and as a disease entity of concern to practitioners in the same state.[40] In 1967, BTV was biologically transmitted under experimental conditions by bites of *C. variipennis* from sheep to sheep, sheep to cattle, cattle to cattle, and cattle to sheep.[25] Vertical or transplacental transmission in cattle was demonstrated in 1965 when BTV was isolated from blood of a nursing beef breed calf with extensive necrotic oral lesions. The calf was born in Idaho on December 25, 1964, and the blood sample was obtained in March, 1965. Winter temperatures during this period precluded the presence of adult vectors. This strongly implicated the *in utero* infection.[41] These data stimulated a series of studies on BTV in pregnant cattle infected by bites of the vector. These studies have proven that BTV causes infections of extended duration, is abortogenic and teratogenic, crosses the placenta and infects the fetus, and produces reservoirs of BTV in some calves that are immunologically non-responsive.[41 42 43] Thus, there is little doubt that cattle play a major role in the perpetuation of BTV.

1. *Carrier Cattle.*

Carrier cattle develop persisting infections and may be immunologically nonresponsive. Upon infection with virulent BTV, cattle develop a high titering viremia that is long lasting. There is great potential for the coexistence of BTV and its specific neutralizing antibody.[13] Carrier cattle are more likely to result from transplacental transmission of BTV to the fetus irrespective of the route of infection of the

dam (vector bite or semen).[41 44] The BTV carrier state has been experimentally demonstrated in healthy cattle,[45] and after natural field infections of cattle,[46] of elk calves born to dams exposed to BTV in early gestation,[47] and of free-ranging white-tailed deer.[48] Unfortunately, carrier animals are often difficult to detect by virus isolation procedures and that fact accounts for the various negative statements made by some scientists. Bovine practitioners should recognize that female cattle impregnated in the fall when BTV is the most active in an enzootic or new incursion area have a potential to transmit BTV to their fetus in early gestation. Under these circumstances (usually under 150 days of gestation) the fetus may not be killed by BTV infection but may be born alive. Virus may be present in its blood, and humoral antibody may not be detectable. These live calves are then immunologically nonresponsive to the virus with the currently available tests. The nonresponsive state may be lost at about 2 years of age to the homologous virus with a fatal consequence,[43] but may also be lost shortly after birth in response to infection with a heterologous BTV serotype. The congenitally infected calf would develop group specific BTV antibodies and serotype specific neutralizing antibodies to the heterologous BTV serotype only. Passively acquired BTV antibody may complicate the situation for newborn calves.[14]

2. *Reproductive consequences.*

Many bovine practitioners are already familiar with some reproductive consequences of BTV infection through direct experience. However, the field evidence is thought to be circumstantial or anecdotal by some scientists because it was obtained retrospectively after other possible etiologic agents were ruled out and because only a high BTV or EHDV seroconversion rate was found in the infected cattle. BTV or EHDV have been isolated from dams and fetuses or neonates on numerous occasions in association with reproductive problems. A listing of the reproductive consequences of BTV infection includes anestrus, infertility (often due to silent abortions), abortions that include "abortion storms", mummified fetuses and stillborn calves. Liveborn calves may exhibit developmental anomalies and dysfunctions that include transient blindness, ataxia, weakness, and poor growth characteristics. The variability in anomalies observed probably are a result of BTV concentration, virulence of the virus, and gestational age of the fetus. The more common types of anomalies include excessive gingival tissue, arthrogryposis (crooked leg syndrome) which may involve one or all 4 limbs, skeletal defects, and hydranencephaly.[29 41 49 50 51] In one field situation involving known BTV infection of pregnant cattle, there was a 1/3 reduction of size and weight of the newborn calves as compared to calves born in previous years. In another known BTV infected herd that experienced an "abortion storm" in the fall, less than 50% of the calf crop survived their anomalies and dysfunctions. Of calves that survive congenitally acquired infection, some may be unthrifty and possible carriers of BTV. BTV isolation attempts on individual surviving calves in such a herd is feasible at present. More field data and follow-up data on BT and other possible concurrent infections are urgently needed to properly evaluate the significance and impact of these infections and to make recommendations toward their prevention and control.

The presence of BTV in semen of infected bulls[52 53 54 55 56] has prompted considerable anxiety in the artificial insemination (AI) industry[57] even though a number of other viruses are known to be shed in bull semen.[58] This is because of the problems associated with the importation of bovine semen into countries considered free of BT. The virus was first isolated from semen of a BTV carrier bull in 1972;[52] when this bull was used in a natural breeding experiment 3 years later, BTV was transmitted by 3 natural services per heifer to all 14 heifers in the experiment.[44] By viral isolation procedures in embryonated chicken eggs, sheep, and cell cultures over a period of 11 years, BTV was only isolated in about 26% of the 389 samples tested.[45] The male offspring of this BTV carrier bull were found to sporadically shed BTV in their semen. Naturally infected bulls have also been found to shed BTV in their semen,[54] but information was lacking on AI of cows with BTV-contaminated semen until recently.[59] These AI data showed that 6 of 9 heifers inseminated with one straw of BTV-infected semen became pregnant, while 3 of 9 became viremic and developed antibody to BTV. A fourth heifer seroconverted but was not detectably viremic. The semen had been collected during the course of the bull's experimental infection and it was later shown to contain titratable quantities of BTV.[59] No evidence of fetal infection was found in the heifer that became infected and pregnant, or in the other 5 heifers that were pregnant but were not apparently infected. **In the authors' opinion there is no longer a question on the presence of BTV in semen but rather, what are the consequences of the virus presence and under what circumstances does viral shedding occur.** The carrier bull at the Denver Laboratory still has BTV in his semen after 13 years of age. As isolation procedures for BTV in semen improve and more scientists gain expertise in viral isolation throughout the world, detection of BTV in semen may become common place. The authors believe that testes are an ideal location for BTV perpetuation because BTV is best isolated in embryonating chicken eggs held at 33.5C rather than at 37C; the testes are 7 to 8C lower than a bull's normal body temperature. Despite BTV humoral antibodies in the carrier bull's blood, similar antibodies have never been found in his seminal plasma.[45]

A number of bovine practitioners are involved in bovine embryo transfer work as an alternative to movement of live cattle for the transfer of selected germplasm nationally and internationally. Naturally, the question of BTV transmission by this method usually arises and information was recently published suggesting that transmission of BTV

under standard embryo transfer conditions from infected donors to uninfected recipients **was unlikely to occur.**[60] The embryos were recovered nonsurgically from 20 donor cattle during their peak viremia. These recovered embryos were then surgically transferred to BTV seronegative recipients 7 to 8 or 10 to 11 days after donor estrus and 21 of 39 recipients became pregnant. More importantly, BTV was not isolated from the blood of any recipient and none of them seroconverted to BTV after 60 days following the transfer.

D. *"Overwintering" of BTV.*

An effective mechanism for the perpetuation of BTV in nature is one that would maintain the virus indefinitely in a geographic area, especially in temperate regions where low winter temperatures preclude flying insect activity and transmission by the biting gnat. The gnat overwinters in the larval stage (egg-larvae-pupae-adult) and to date there is no evidence to suggest transovarial transmission or that larvae will harbor BTV.[61 62] Thus, a reservoir vertebrate host such as carrier cattle is a likely possibility for BTV to "overwinter." If cattle are the reservour hosts for BTV, then a mechanism for virus escape from the host that may or may not have BTV antibody must exist. Such a mechanism involving a healthy BTV carrier bull and biting gnats was described.[63] The biological mechanism was mediated by the bites of non-infected gnats. Initial bites of the gnat stimulated the release of BTV into the circulation within hours of insect feeding. Other non-infected gnats that fed several hours later on the same bull and were properly incubated for 14 to 21 days, became infected and transmitted BTV upon feeding on BTV susceptible sheep. Although this biting gnat-mediated biological recovery of BTV was originally demonstrated on an experimentally infected individual bovine, the sequence of events has been demonstrated for other carrier cattle (field and experimental sources)[46] and for elk.[47] In most situations a dramatic increase in viremia does not occur but the viremia becomes detectable. As might be expected, the efficiency of this biological recovery mechanism is less than 50%. Studies are underway to better define and explain the parameters involved in the mechanism. Nonetheless, the phenomenon occurs and under the vast resevoir potential of nature there need only be a few BTV carrier cattle and competent biting gnat populations available to allow BTV perpetuation in many geographic areas.

E. *Dual or multiple infections.*

As mentioned earlier there are now 23 serotypes of BTV in the world; 5 are present in the U.S. (serotypes 2, 10,11, 13, 17). One serotype is only found in the U.S. (serotype 17). Because of limited and variable cross protection among these various BTV serotypes in animals, there is a theoretical potential of multiple infections for cattle in the U.S. Although this fact in itself is important to the bovine practitioner, a concurrent, dual or multiple serotypic infection in cattle represents a greater potential danger to the cattle industry. Of primary concern is the fact that concurrent dual serotypic infections may be common. They have been reported under field conditions in both California[11] and Colorado,[10] with severe clinical illness being observed in one instance.

Both BTV and EHDV (orbivirus genus) have a double-stranded ribonucleic acid (RNA) genome as the genetic and infectivity component. This genetic material exists as 10 RNA segments and each single segment codes for a specific protein. Simplistically, during the infection process of cells with BTV particles, the infectious RNA genome is eventually freed within a cell where it becomes available for its replication cycle (multiplication and growth phase). Other cells are subsequently infected. In dual or multiple infections of cattle by two or more serotypes of BTV there is a period of time when the 10 RNA segments, representative of 10 proteins including the antigenic determinants, are free inside the cell. At this moment there is a potential for the reassortment and recombination of the parental genome segments. For example, if a bovine animal is concurrently infected with BTV serotype 11 and 17 some of the new progeny BTV particles may actually be a mixture of these 2 serotypes. This could lead to the evolution of a new BTV strain with a unique genetic makeup. The reassortment process has been shown to occur under experimental cell culture conditions[64 65 66] and, therefore, when either a bovine animal or the biting gnat is concurrently infected with multiple serotypes of virus, there is an opportunity for reassortment to occur. **Each bovine practitioner should be aware of reassortment and recombination because extensive use of multivalent modified live BTV vaccines might create a greater hazard than that which already exists. This is the primary reason why the authors believe that an effective killed BTV vaccine must be developed for the safe use of vaccines for the control of BTV in problem areas.**

Summary

Bluetonge (BT) is a complex disease that involves a wide host range of domestic and wild ruminants. These hosts vary in susceptibility. A plurality of virus serotypes with the ability to coexist with specific neutralizing antibodies in many of the host animals occurs. The primary vector in the U.S., *Culicoides variipennis,* is highly adaptable to its environment. The vector, commonly known as a biting gnat, will blood feed on many ruminant animals, is normally competent to transmit the virus, and when infected, it harbors high concentrations of the virus for life. The serologic and virologic diagnosis of BT in cattle is difficult. The vector transmission, plurality of BTV serotypes, wide animal host range, reservoir cattle, overwintering, and multiple infection aspects were discussed. **In conclusion, BT is an important infectious disease of cattle.**

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Abstracts

Conception Rates in Cows After Various Synchronisation Techniques Using Progesterone Releasing Intravaginal Devices, Y. Folman, S. R. McPhee, I. A. Cumming, I. F. Davis, and W. A. Chamley, Aust. Vet. J. 60-44.

The effects of different treatments for oestrus synchronisation on the incidence of oestrus and fertility levels in dairy cows were studied in 2 experiments.

In Experiment 1, 200 lactating cows were allotted to 5 groups and the treatments imposed were either: 1. Untreated controls, 2. An injection of 0.5 mg of cloprostenol followed 13 days later by a progesterone releasing intravaginal device (PRID) inserted for 12 days, 3. A PRID, with a capsule containing 10mg of oestradiol benzoate (ODB) attached, inserted for 12 days, 4. A PRID inserted for 12 days with 0.5 mg of cloprostenol administered 24 h before PRID removal or, 5. As for 4 but 14 days after fixed-time insemination a second PRID was inserted for 12 days. Treated cows were inseminated 56 h after PRID removal and at an observed oestrus during the subsequent 30 days. The control group was inseminated at an observed oestrus during this 30-day period. For treatments 2, 3, 4 and 5, respectively, the percentage of cows showing oestrus by 60 h after PRID removal was 70, 40, 67 and 43 and conception rates to the fixed time insemination were 34, 33, 49 and 29%. Calving rates of cows inseminated at an observed oestrus during a 30-day period were 70, 75, 70, 83 and 82% for treatments 1, 2, 3, 4 and 5, respectively.

In Experiment 2, 60 lactating cows were divided into 2 groups and the treatments imposed were either 1: An injection of 0.5 mg of cloprostenol followed 13 days later by a PRID inserted for 12 days or 2: As for 1 but 14 days after fixed-time insemination a second PRID was inserted for 12 days. Treated cows were inseminated 56 h after PRID removal and at an observed oestrus over a period from the first insemination to 6 days after removal of the second PRID. For treatments 1 and 2, respectively, 73 and 71% of cows showed oestrus by 60 h after removal of the first PRID and 40% and 46% conveived to the fixed time insemination. The conception rates to inseminations over the treatment period were 73 and 70% for treatments 1 and 2, respectively.

None of the treatments resulted in conception rates which were lower than those of control cows provided that treated cows were reinseminated at observed oestrus. Treatment 4 provided the most practicable technique for oestrus synchronization.

The Effect of Escherichia coli Endotoxin and Culture Filtrate on the Lactating Bovine Mammary Gland. A. J. Frost, B. E. Brooker, and A. W. Hill, Aust. Vet. J., 61:77-82.

The pathogenesis of coliform mastitis was studied by observing pathological changes in lactating glands after infusion of either endotoxin or the sterile culture filtrate (CCF) of the medium in which *Escherichia coli* strain B117 had been grown. Both infusions produced a rapid and intense inflammatory response by 4 h with a marked increase of serum proteins in the milk. Before dispersing into the milk, neutrophils were attached to the ductular epithelium; highest cell counts in the milk were recorded when the tissue reaction had waned. Oedema of the ductular epithelium had occurred, particularly where neutrophils were actively migrating. The infusion of CCF produced, in addition to inflammation, degeneration and necrosis of ductular cells. The smallest lesions healed very rapidly. There was evidence of differing cell susceptibility to the necrotising toxin as well as uneven distribution over the epithelial surface. All changes observed were confined to the regions of the teat and lactiferous sinuses with little effect on the secreting tissue. The role of the necrotising toxin in the natural disease remains undetermined.

Experimental Production of Fatal Mucosal Disease in Cattle, J. Brownlie, M. C. Clarke, C. J. Howard, Veterinary Record (1984) 114, 535-536.

Three outbreaks of mucosal disease were investigated. Careful examination of 47 cattle that were persistently viraemic with bovine virus (BVDV) revealed no clinical disease, no or low levels of BVDV antibody and only non-cytopathic virus in their blood. The four animals with mucosal disease all showed clinical disease and both cytopathic and non-cytopathic virus in their blood. Following post mortem examination, there were particularly high levels of cytopathic virus in gut tissue. A hypothesis for the induction of mucosal disease is suggested. It states that animals become persistently infected with non-cytopathic virus following in utero infection and when, in post natal life, they become superinfected with a cytopathic virus, then mucosal disease ensues. The experimental reproduction of mucosal disease in support of this hypothesis is described.