

Congenital Anomalies in Cattle Associated with an Epizootic of Bluetongue Virus (Serotype II)

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Summary

An epizootic of bluetongue occurred in several ruminant species in Oklahoma between December, 1983, and May, 1984. Of 20 clinical specimens from cattle fetuses and neonates examined for bluetongue virus (BTV), virus was isolated from 9 specimens. Five of the 9 viral isolates were serotyped as BTV 11. Serotype 11 of BTV was also identified in 2 sheep fetuses and in neonates of an Addax, a Nubian ibex, a Cape buffalo and a Sable antelope. Serotype 17 of BTV was identified in a sheep neonate. The ability to isolate BTV from clinical specimens was enhanced by increasing the adsorption time of virus onto cell cultures. An increase in congenital anomalies in cattle was associated with the epizootic of BTV. The most prominent histopathic lesion of BTV was found in the brain of the affected neonates or fetuses.

Introduction

Bluetongue is a disease principally of sheep, although in bluetongue-affected cattle, fever, nasal discharge, erosions and necrosis of the oral mucosa, edema, scab formation on teats, pityriasis, coronitis and lameness resulting from laminitis are produced (1,2,3,4). However, infection in the adult bovine is usually inapparent (1,3,5). In the United States, 4 viral serotypes of bluetongue virus (BTV) are presently known; namely, 10, 11, 13 and 17 (1,6,7). Recently, serotype 2 of BTV has been documented in a cattle herd in Florida (8).

Bluetongue virus has been described as abortogenic and teratogenic in ruminants (1,2). Cattle (pregnant heifers) experimentally-infected with BTV have aborted and/or given birth to stillborn calves (2). Congenital anomalies reported for BTV-infected fetuses are arthrogryposis, agnathia, prognathia with domed cranium and incisors covered 2/3 or 3/4 with gingival tissue (1,2,7). The hydranencephaly seen in BTV-affected animals has been reported as the cause of "dummy" calves (1,2,8). Sheep infected with BTV have brain lesions described as hydrocephalies and porencephaly (1,9,10,11) with associated fetal malformations (12). The stage of gestation at which the sheep or bovine fetus is infected appears to play a vital role in the pathogenesis of a BTV infections (9,10,13,14).

The purpose of this report is to describe an epizootic of bluetongue during which an increased number of congenital anomalies were reported in cattle in Oklahoma. During this epizootic period, other ruminant species were observed with clinical signs of bluetongue. We also describe the isolation and serologic identification of the viral isolates of BTV from neonates or fetuses obtained from several of these ruminant species.

Materials and Methods

Clinical Histories

Case No. 1: A cow, an Angus-Chianiana cross, aborted a 6-month old fetus. This cow was in a herd of 250 cows which had had 7 abortions within a period of 1 month. The fetus was sent for a diagnostic evaluation to the Oklahoma Animal Disease Diagnostic Laboratory.

Case No. 2: A 1-day old dead calf was received from a Holstein herd of 200 cows in which 3 calf deaths had been recorded. The calves were born small, weak and unable to stand. All the calves had died within 24 hours after birth.

Case No. 3: A bovine neonate from a herd of Herefords was received in the laboratory. The calf was incoordinated and had an arthrogryposis of all 4 limbs. Opisthotonus was also seen in the calf prior to euthanasia.

Case No. 4: A fetus was sent to the laboratory from a herd of Herefords which consisted of 100 cows. Within a period of 6 weeks, 7 to 10 abortions had occurred within the herd. All the adult cows were healthy.

Case No. 5: A neonate Brahman calf was received from a herd with 29 cows. The calf had died within a few hours following birth. A total of 6 dead calves had been born within the herd; however, no fetal or neonatal malformations had been seen.

Clinical Specimens:

From December, 1983, to May, 1984, a total of 20 specimens from bovine fetuses or neonates were examined for BTV. The clinical specimens included the following tissues: spleen, bone marrow, lymph nodes, lung, kidney and heparinized blood. A similar assortment of tissues were examined for other ruminant species tested.

The tissues were prepared as a 20% w/v suspension by homogenization in F15 medium^a which contained buffered lactose peptone (1:1). The centrifuged homogenates were stored at -20 C until they were inoculated into eggs. Erythrocytes and buffy coat cells were washed 3 times in sterile phosphate-buffered saline and disrupted in medium which contained buffered lactose peptone.

Procedure for Viral Isolation and Identification:

An inoculum of 0.1 ml was given intravenously into 9 to 12 day old embryonated chicken eggs. For each clinical specimen 4 to 6 eggs were used and incubated at 33 C. The eggs were placed in the refrigerator after death and prior to removal of embryos. Chicken embryos which exhibited a cherry-red coloration and/or edema were considered positive or suspicious for BTV. Chicken embryos that died within 24 hours after inoculation were not used as they were considered to have died of trauma. The embryonic tissues of affected eggs were homogenized then centrifuged at 800 xg and subsequently inoculated onto cell cultures.

One to 2 day old African green monkey kidney Vero-M (Maru) cells grown in 25 cm² flasks were used for the inoculation of egg-passaged materials. The cells were grown in minimum essential medium (Eagles) F15 medium which contained 200 ug/ml of gentamicin and 5% fetal bovine serum (FBS). The specimens were adsorbed onto Vero-M cells using 1 ml of embryonic homogenate with 1 ml of medium which contained 10 mM of HEPES buffer.

Flasks which contained the inoculated cell monolayer were tightly sealed and placed onto a rocker plate for overnight adsorption. Following viral adsorption, the inoculum was removed from each flask and cells were refed with 5 ml of F15 medium containing 5% FBS and 200 ug/ml of gentamicin. The inoculated flasks were placed in a CO₂ incubator set at 32-33 C. Each specimen was passaged 5 to 6 times in cell culture before it was considered negative. Flasks with viral cytopathic effects, after the addition of 5 ml of buffered lactose peptone, were harvested by 3 cycles of freeze-thaw.

Serotyping of BTV:

Aliquots of each viral isolate were sent frozen to the Arthropod-borne Animal Disease Laboratory for serotyping by the plaque-inhibition method (15).

Results

A total of 20 specimens from cattle with a clinical history compatible with bluetongue infection were examined during the epizootic. Nine isolations of bluetongue virus were made of which 5 have been serotyped and 4 are pending serologic identification.*

^aKC Biologicals, Lenexa, Kansas 66215.

*results of the BTV 4 isolated have since shown to be serotype 11

From the 5 clinical specimens derived from bovine neonates or fetuses in which BTV was isolated, serotype 11 was identified in each case. The clinical history and diagnostic profiles on each case are presented in Table 1. In cases numbered 2 and 3, a cerebellar hypoplasia was seen in the brain examined. In addition, the neonate in case No. 3 was a hydrocephalic calf. This neonate calf also had a porencephaly or cavitations in the brain. Porencephaly (Figure 1) was also observed in sections of brain from case No. 5, a Brahman neonate.

However, several bovine cases of suspected bluetongue were examined which had gross lesions characteristic of a bluetongue infection, but BTV was not isolated from these animals. The lesions seen are presented in Figures 2, 3, 4 and 5. Bluetongue virus was not isolated from the pictured animal; however in animals where similar lesions were seen, BTV was isolated but not serotyped. The lesions seen in most BTV-affected bovines included: lack of retraction of gingival tissue (Figure 2) over the incisors, malformations of the vertebral column, i.e. scoliosis and kyphosis (Figures 3 and 4) and atresia ani (Figure 5).

During this epizootic of bluetongue, other ruminant species were found to be infected with BTV. In Table 2, we present the isolation and identification of BTV from sheep during the epizootic. In 2 cases, the BTV serotype was 11; however, in 1 sheep fetus serotype 17 was identified.

In exotic ruminants located at a zoologic park in Oklahoma City and examined for bluetongue infection during this period, BTV was identified in a 1-day old Cape buffalo, *Syncerus caffer caffer*, a 1-day old Nubian ibex, *Capra ibex nubiana*; a 1-month old addax, *Addax nasomaculatus* and a 1-day old sable antelope, *Hippotragus niger*. In each case the BTV isolated was serotype 11.

Discussion

The epizootiology of BTV in cattle has been documented primarily by serologic surveys of cattle herds (16) and slaughter cattle (17). A serologic survey of sera from sheep, goats, wild bighorn sheep and white-tailed deer also indicated the presence of BTV in these latter species (17). Bluetongue has also been identified in exotic ruminants such as the muntjac (*Muntiacus reevesi*) and kudu (*Tragelaphus capensis*) (18). In 1973, a study of the frequency of BTV serotypes in the U.S. indicated that serotypes 11 and 17 occurred with greater frequency than serotypes 10 and 13 (6). Recently, Kocan et al. (19) have reported the inapparent infection of white-tailed deer in Oklahoma from which BTV serotypes 13 and 17 have been isolated.

In 1982-83 in Colorado, BTV was isolated from a large number of ruminants; namely, cattle, sheep and goats, and the major BTV serotype found was 11^b. In 1980, the number

^bDr. T. L. Barber, Arthropod-borne Animal Disease Laboratory, Denver, Colorado: Personal communication, 1984.

TABLE 1. History and Diagnostic Profile on Bovines Positive for Bluetongue Virus.

Case No.	No. of Cattle In Herd	Breed	Age	Clinical History	Histopathology	Serology for BTV	BTV Serotype Isolated
1	250	Angus-Chianiana Cross	Fetus	Abortions	Bronchitis Hepatitis	N.D.	11
2	200	Holstein	1 day	Weak Calves	Brain Lesions	N	11
3	N.A.	Hereford	1 day	Arthrogryposis	Porencephaly	N	11
4	100	Hereford	Fetus	Abortion	Renal Hypoplasia; Osteodystrophy of Mandible	N.D.*	11
5	29	Brahman	1 day	Weak Calves	Porencephaly	N	11

* Cows (5) in herd tested; 2 were sero-positive and 3 were sero-negative.
N.A. = not available; N.D. = not done; N = negative.

Figure 1. Cavitations (c) present (arrows) in white matter of cerebrum from a neonate calf described in Case No. 3. The histopathologic lesion in the brain was described as porencephaly. X 50.

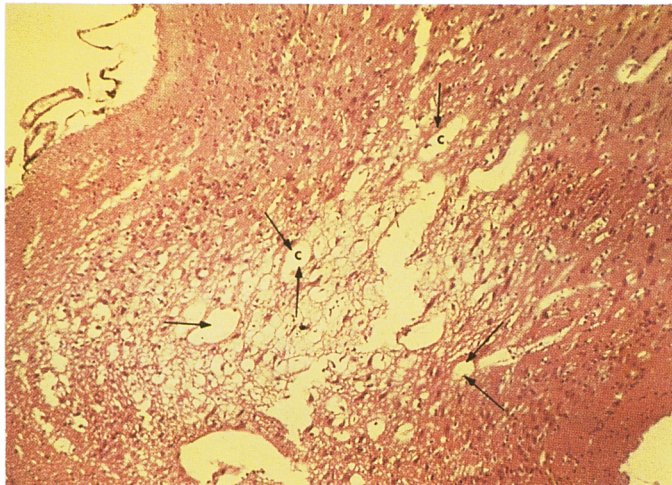


Figure 2. Lack of retraction of gingival tissue over incisors (arrows) of a bovine neonate.



Figure 3. Red Angus neonate with congenital anomalies of vertebral column and a slightly domed head.



of BTV-seropositive cattle in Oklahoma was reported to be 68.6% (7).

Certain congenital anomalies and some abortions seen in cattle have been described as a sequela to BTV infection (5). Congenital infection early in gestation appears to give rise to BTV persistently-infected calves (14). However in this preceding study, early infection did not lead to immunologic tolerance or persistence of virus post-natally. The gross lesions seen with developmental anomalies associated with bluetongue infection have been previously described (2,3,7). However, the histopathic lesions seen in the brain were first described in sheep as an hydranencephaly with cavitations in the brain cerebrum and cerebellum (9,10,11). A hydrocephalus and hypoplasia of the brain of sheep have also been described (12). The most descriptive pathologic lesions in the brain of BTV-infected ruminants have been

Figure 4. Lateral view of spinal column from Red Angus neonate depicted in Figure 3. Kyphosis (arrow-head) and scoliosis of spine (arrow) was present. A vertebral body is encircled. s = spinal cord.

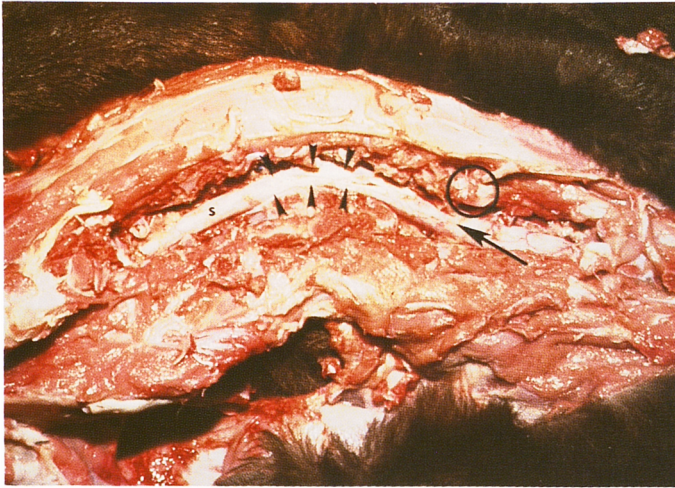
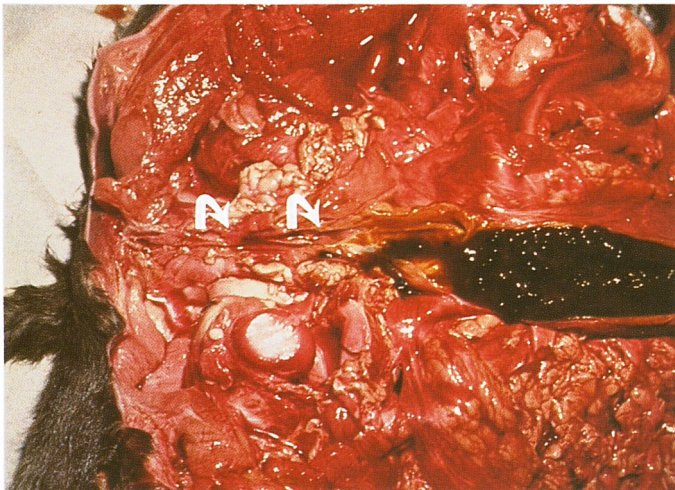


Figure 5. Atresia ani (arrows) found in Red Angus neonate depicted in Figure 3.



described in sheep (9,10,11,20). These lesions included: a necrotizing encephalitis, porencephaly, microcavitations and hydranencephaly (8,9,10,11). Similar cavitory lesions in the cerebral hemispheres have been described in BTV-infected cattle (8,13). The study of the pathogenesis of the brain lesions in cattle indicated that the brain of the bovine fetus is most susceptible to BTV at 125 days into gestation (13). However, hydranencephaly with porencephalic cavitation of the cerebellum in calves has been associated with an infection by bovine viral diarrhea virus (21).

Studies have shown that BTV can coexist with high levels of serum neutralizing antibodies (2, 3). Furthermore, dual infections with serotype 11 and 17 have been described in both sheep and cattle (22). An infection in a host by dual serotypes of BTV may lead to recombinants of BTV which may alter the biologic properties of the virus or give rise to new serotypes (22).

During the period of December, 1983, to May, 1984, an epizootic of BTV (serotype 11) was documented in cattle, sheep and exotic ruminants located in Oklahoma. Although the viral lesions were minimal in adults, an increase in congenital anomalies were seen in neonates and fetuses from affected pregnant cattle. The gross lesions seen have been previously described in cattle (2,3,7). Most of the cattle fetuses or neonates from which BTV was isolated had lesions in the brain which were manifested as microcavitation in the cerebrum or cerebellum. The histopathic lesions indicated the presence of porencephaly in many of the brains obtained from BTV-affected bovines. Of interest was the number of other ruminants affected with BTV during this epizootic. The serotype of BTV identified was 11 in all species, except the presence of serotype 17 in a sheep neonate. In a comparison of genomes of different isolates of BTV, Knudson et. al. (23) found differences in the double-stranded RNA of 2 isolates of serotype 11. The implication of this finding is that the same viral serotype may exhibit different biologic properties such as pathogenicity, host range and immunogenicity (23).

In a recent study (24) no fetal infection, as measured by isolation of virus, or anomalies occurred in BTV-infected

TABLE 2. History and Diagnostic Profile on Sheep Positive for Bluetongue Virus.

Case No.	No. of Cattle In Herd	Breed	Age	Clinical History	Histopathology	BTV Serotype Isolated
1	45	Suffolk	2 Years	N.A.	N.D.	11
2	20	Suffolk	1 day	Malformations; Deformed Fetuses	Cerebral, cerebellar hypoplasia	17
3	15	Suffolk	Fetus	Stillborn abortions	N.A.	11

N.D. = Not done; N.A. = Not Available.

pregnant heifers following insemination of virus-containing bovine semen. Although the number of BTV-inoculated heifers was small in the preceding study (24), it does suggest a low level of infection of bovine fetuses by BTV.

The ability of the laboratory to isolate BTV in cell cultures from these BTV-affected animals was enhanced by the extended period of adsorption of virus onto cells.

The observation of fetal anomalies in herds of cattle should alert veterinarians to the possible presence of BTV and a laboratory diagnosis for BTV should be conducted on the suspect herd.

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