Incursion of bluetongue virus into the Okanagan Valley, British Columbia

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Abstract — Bluetongue virus was isolated from a sentinel herd in British Columbia. Virus isolation was by intravenous inoculation of embryonated chicken eggs and subculture in BHK-21 cells. The cytopathic agent was identified as bluetongue virus by electron microscopy and the immunoperoxidase test. The serotype was identified as serotype 11 by virus neutralization.

Résumé — Incursion du virus de la fièvre catarrhale dans La Vallée de l'Okanagan en Colombie-Britannique. Le virus de la fièvre catarrhale a été isolé chez un troupeau sentinelle en Colombie-Britannique. L'isolation du virus s'est fait par inoculation intraveineuse d'œufs de poules embryonnés et subculture dans les cellules BHK-21. L'agent cytopathique a été identifié comme étant le virus de la fièvre catarrhale par microscopie électronique et immunoperoxydase. Le sérotype 11 a été identifié par neutralisation virale.

(Traduit par docteur André Blouin)

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Bluetongue (BT) is an insect-transmitted, noncontagious viral disease of domestic and wild ruminants (1). Bluetongue virus (BTV) infection is endemic throughout much of the world. The major economic impact of BTV infection is that BT-free countries may restrict or ban the importation of ruminants and their genetic products from areas of the world in which BTV infection is endemic (2).

The first documented evidence of BTV infection in Canadian cattle was in 1975 on a ranch in British Columbia. Antibodies were detected by a complement fixation test during a routine survey of cattle imported from the United States. The ranch was situated on the Canada-United States border, at the southern end of the Okanagan Valley of British Columbia (3). In October 1987, BTV was isolated from cows showing clinical signs of BTV infection (4). In the following year, BTV serotype 11 was isolated from sentinel cattle in the same area (5). There has been no evidence of incursions since that time, based on both active and passive surveillance. Active surveillance has consisted of a sentinel herd program in the Okanagan Valley and national surveys in 1991 and 1994 (Kellar JA, personal communication). This paper reports a new incursion of BTV type 11 in the Okanagan Valley.

During BTV-infection surveillance in October 1998, serum antibody to BTV was detected by competitive enzyme-linked immunosorbent assay (C-ELISA) (6) in 2 cattle in a sentinel herd in the district of Osoyoos in the Okanagan Valley. Blood samples had been taken from the herd on September 15, 1998, and tested negative for BTV in the C-ELISA test. One sample taken from an animal on October 13 was found to be positive. The herd, including the seropositive animal, was resampled on November 12. On November 18, the initial result on the seropositive animal was confirmed and an

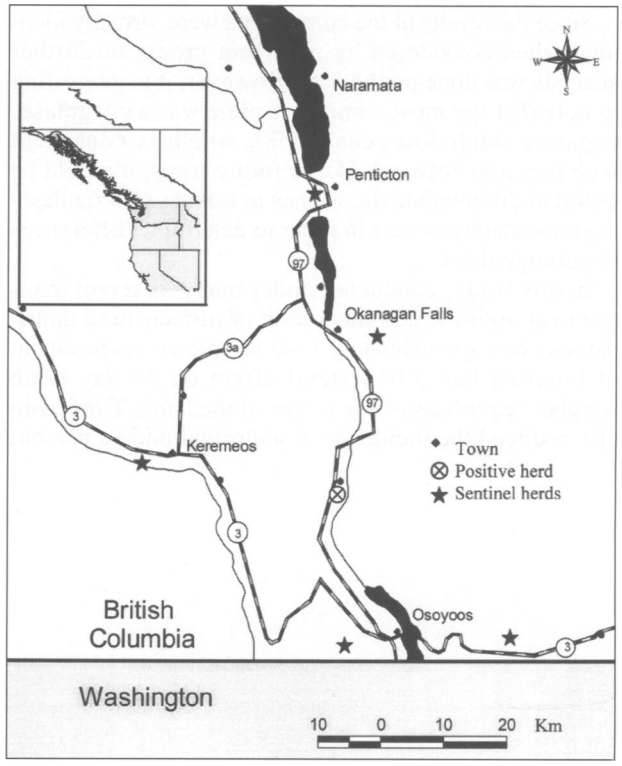

Figure 1. Localization map of the Okanagan Valley, British Columbia. The location of the sentinel herds and the site where BTV-11 activity was detected are indicated.

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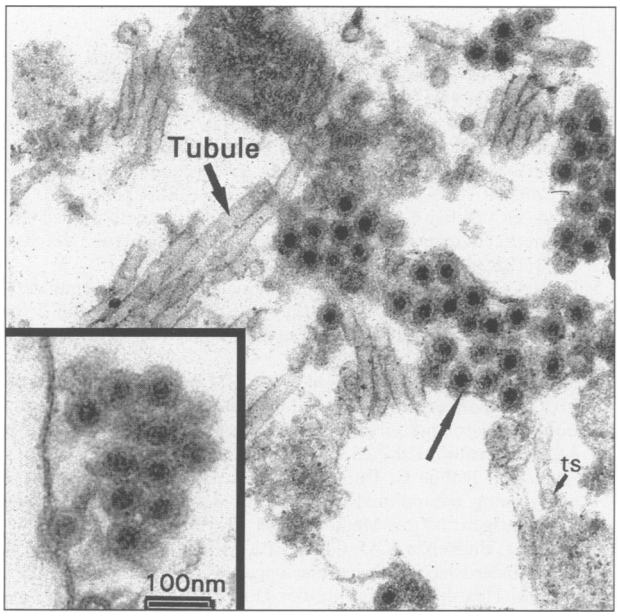

Figure 2. Transmission electron micrograph of an infected cell showing virions and virus specific tubule (Tubule). Tubules are also visible in transverse section (ts). Inset — double-shelled particles at higher magnification.

additional seropositive animal was reported. Blood samples were taken for virus isolation from the 1st and 2nd seropositive animals on November 4 and 24, respectively. An additional sample from the 2nd animal was taken on December 2. No clinical signs of BT were reported in any animals in the affected herd. There was no significant die-off in the surrounding deer population (Canadian Food Inspection Agency (CFIA) staff, personal communication).

For virus isolation, washed red blood cells (RBC) were inoculated, IV, into 10 embryonated chicken eggs (ECE), which were then incubated for 7 d at 33.5°C. Selected embryonic tissues (brain, liver, spleen, heart, and lung) were collected. Suspensions of the embryonic tissues were used for virus isolation in BHK-21 cell cultures.

The BHK-21 cells showing cytopathic effects (CPE) were tested by the BTV indirect immunoperoxidase assay (IPA) (7) and by electron microscopy, in order to identify the isolated cytopathic agent. Results of the IPA revealed the presence of BTV-specific staining in the cytoplasm of the infected cells. Observation by electron microscopy of infected BHK-21 cells indicated the presence of numerous viral particles with characteristic orbivirus morphology.

Extensive CPE was observed in infected cells. The ultrastructure showed that most of the cells were lysed. Large aggregates of double-shelled, spherical virus particles, about 80 nm in diameter, were evident (Figure 2). The virus particles appeared to have a dense core about 60–70 nm in diameter, surrounded by a less dense outer shell. Virions were frequently associated with the cytoskeleton of lysed cells, and many were enclosed within inclusion bodies in the cytoplasm. The infected cells also contained extensive networks of tubules about 55 nm in diameter, visible both longitudinally and in transverse section. None of these features were apparent in the control uninoculated cell cultures.

The results of IPA and electron microscopy suggested that the cytopathic agent was BTV. This virus was designated Okanagan virus isolate/1998 (OKVI/98). A virus neutralization (VN) test was used to type the BTV isolate by using reference sera to all 24 known serotypes of BTV, as previously described (8). This isolate (OKVI/98) was neutralized by anti-BTV 11 serum.

The sentinel cattle from which BTV was isolated were located approximately 24 km north of the 49th parallel and about 8 km south of the city of Oliver (Figure 1). Canada is considered free of BTV, except in a small area of British Columbia, where all the incursions of BTV have occurred. This area or zone in the Okanagan Valley was defined as the area enclosed by a line extending northward from 49° latitude and 120° 15′ longitude to 50° 30′ latitude and 119° 35′ longitude. From there, the line extends eastward to 50° 45′ latitude and 119° longitude, and then southward to 49° latitude and 118° 15′ longitude (9).

Canada's official BT policy, established in 1988, delineated the zone described above. It implemented a surveillance mechanism for determining BTV seroprevalence within the zone by using a sentinel herd program, and established an animal identification strategy for all animals leaving the Okanagan Valley. The sentinel herd program has 6 herds of cattle, consisting of 7 animals per herd, which are distributed on or within 48 km of the Canada-United States border (Figure 1). The herds are screened in early April, prior to the vector season, to ensure that all animals are seronegative for BTV. Blood sampling is performed every 3 wk throughout the summer, and continues until the first severe frost, in early to mid-October. After this time, the vector *Culicoides* is no longer present. All susceptible animals leaving the Okanagan Valley are identified, so that they can be traced back to the valley in the event that a BTV reactor shows up in another region of Canada.

The sentinel herd and animal identification program were designed to provide and maintain strong scientific evidence to international trading partners that the occurrence of seroreactors or virus isolation from animals in this specific zone did not provide evidence to support Canada-wide international trade restrictions. Combined with national seroprevalence surveys, conducted approximately every 3 y, the sentinel herd program and animal identification strategy provide strong evidence to support Canada's policy and position.

After 10 y of surveillance, BTV-11 has now been isolated on 2 occasions from cattle in Canada. In 1976, BTV infection was detected only serologically and there were no reports of clinical signs of the disease (3). This is in contrast to the outbreaks of 1987 and 1988, where there were clinical signs of disease in cattle and sheep and virus was isolated from one of the sentinel herds (5). The incursion of BTV in 1998 was more limited than in the previous outbreaks and the use of ECE in combination with tissue culture techniques proved to be the method of choice for isolation and identification of BTV in field specimens.

Based on serological and virological data, the infection of these animals likely occurred at the end of September 1998. The 1st animal tested negative by C-ELISA on September 15 and positive on October 13. If the animal was infected during the last week of September, there would have been enough time for

seroconversion to occur. Infectious virus can be isolated consistently from RBC up to and even beyond 40 d after infection, despite high titers of neutralizing antibodies in serum (10).

These findings are compatible with the epidemiology of BT in this area of British Columbia. In the 1987 and 1988 outbreaks, the first isolation of BTV-11 was from samples collected in early October (11). It has been suggested that the 1987 and 1988 incursions of BTV resulted from wind-borne infected *Culicoides*, carried north from the western United States. It is hypothesized that a complex association of atmospheric and weather interactions allow infected *Culicoides* to be introduced further north into Canada from the United States (12). Although there is no evidence that this happened in 1988, it is a presumable scenario for the introduction of BTV into the Okanagan Valley.

The identification of these 2 infected animals illustrates that the sentinel herd program is effective. The results of the 1999 national survey, if negative for serological evidence of BTV infection, will again support the contention that the Okanagan Valley is the only area in Canada that experiences sporadic episodes of BTV infection of susceptible domestic animals. Thus, these current infections do not influence the health status of animals in other parts of Canada or alter Canada's international trade position.

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Answers to Quiz Corner/Les réponses du test éclair

1. d — These signs describe proximal enteritis.
   d — Ces signes décrivent l'entérite proximale.
2. c — The femoral nerve innervates the quadriceps femoris muscle.
   c — Le nerf fémoral innerve le muscle quadriceps fémoral.
3. c — The history is consistent with nervous ketosis.
   c — Le cas est compatible avec l'acidose nerveuse.
4. a — Zearalenone does not cause abortion.
   a — La zéaralénone ne cause pas d'avortement.
5. d — Two or more of the described lesions are usually observed in feedlot lambs dying from enterotoxemia.
   d — Deux ou plus des lésions décrites sont habituellement observées chez les agneaux d'engraissement qui meurent à la suite d'entérotoxémie.
6. a — Excessive thyroxine causes secondary cardiac hypertrophy and cardiomyopathy.
   a — L'excès de thyroxine cause une hypertrophie cardiaque secondaire et de la cardiomyopathie.
7. d — Ammonia is converted to nitrite by *Nitrosomonas* and nitrite is converted to nitrate by *Nitrobacter*. *Aeromonas* is a pathogenic bacterium that is ubiquitous in aquatic environments.
   d — L'ammoniaque est transformée en nitrite par Nitrosomonas *et le nitrite est transformé en nitrate par* Nitrobacter. Aeromonas *est une bactérie pathogène ubiquiste dans le milieu aquatique.*
8. d — Metastasis does not usually produce clinical signs until late in the disease.
   d — La métastase ne cause habituellement pas de signes cliniques jusque tard dans l'évolution de la maladie.
9. e — Enzyme-linked immunosorbent assays and the newer immunochromatographic assays detect adult heartworm antigen, not microfilariae. These tests are not affected by the presence or absence of microfilariae.
   e — L'ELISA et le nouveau test de dosage immunochromatographique décèlent l'antigène de Dirofilaria *adulte, mais non les microfilaires. Les résultats de ces tests ne sont pas influencés par la présence ou l'absence de microfilaires.*
10. b — Epinephrine affects glycogenolysis in both muscle and liver. Glucagon can selectively mobilize glucose from hepatic glycogen stores without depleting muscle glycogen.
    b — L'épinéphrine affecte la glycogénolyse dans le muscle et le foie. Le glucagon peut mobiliser de façon sélective le glucose du glycogène hépatique sans provoquer la déplétion du glycogène musculaire.