Evidence for Bluetongue Virus in Canada: 1976-1979

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ABSTRACT

Since the identification of approximately 1 400 bovine serological reactors to bluetongue virus in the Okanagan Valley of British Columbia in 1976, there has been no evidence of virus establishment in Canada. No clinical signs suggestive of bluetongue were observed. It was not possible to demonstrate viral activity at the time the seropositive animals were detected and subsequent serological testing supports the hypothesis that the virus has not become endemic or indeed survived in Canadian cattle populations. This combined with the dramatic reduction in prevalence of serological reactors in the years following the initial slaughter suggests that viral activity occurred in the Okanagan Valley prior to 1976 and disappeared. There has been no evidence for transmissions different from that expected of a classical arbovirus; that is, no evidence of "vertical" transmission.

RÉSUMÉ

Depuis l'identification d'environ 1400 bovins qui possédaient des anticorps à l'endroit du virus de la fièvre catarrhale du mouton, dans la vallée d'Okanagan, en Colombie Britannique, en 1976, on n'a pu déceler l'évidence de l'établissement du virus, au Canada. On n'a pas non plus observé de signes cliniques semblables à ceux de la fièvre catarrhale du mouton. Il s'avéra impossible de démontrer une activité virale, au temps où les animaux séropositifs furent détectés, et les résultats d'épreuves sérologiques ultérieurement. On n'a pas constaté l'évidence de transmissions réussi à survivre et n'est par conséquent pas devenu enzootique, au sein du cheptel bovin, au Canada. Ces constatations, jointes à la réduction drastique des bovins séropositifs, après l'élimination de leurs congénères séropositifs, permettent de penser qu'une activité virale s'exerça dans la vallée d'Okanagan, avant 1976, et qu'elle disparut ultérieurement. On n'a pas constaté l'évidence de transmissions différentes de celles auxquelles on peut s'attendre de la part d'un arbovirus classique, c'est-à-dire qu'on n'a pas constaté de transmission verticale du virus.

INTRODUCTION

Bluetongue (BT) is a widespread infection of ruminant animals which, although usually inapparent, can result in clinical and pathological signs referable to its destruction of vascular endothelial cells. These include hemorrhage, thrombosis, excoriation of "traumatized" areas such as lips, tongue, coronary bands and rumenal pillars, and central nervous system changes in the developing fetus which may be seen immediately postnatally. There is considerable variation in severity of signs observed with species. Cattle tend to be inapparently infected with bluetongue virus (BTV) while white-tailed deer often show severe changes. Sheep and goats seem to fall between these two extremes.

The bluetongue viruses, of which there are currently 20 serotypes recognized, constitute a group within the genus Orbivirus in the *Reoviridae* family. They are arboviruses usually transmitted by *Culicoides* biting flies. 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 on the vector.

Canada has generally been regarded as BTV free in spite of the widespread presence of the virus in many parts of the U.S.A. and the inadvertent introduction prior to 1975 of several seropositive animals into Canada. Since both the vector and susceptible hosts are abundant in Canada, it is apparent that the correct ecological circumstances (i.e. vector numbers and competence, climatic conditions, concentration of ruminant animals, etc) for BTV survival do not exist.

In 1975, during a survey of nearly 10 000 cattle which had been imported from the U.S.A., 261 serological reactors were identified and destroyed. During the testing of contacts of these reactors in 1976, a ranch in British Columbia yielded 221 reactors from 524 animals tested. As a result of this,

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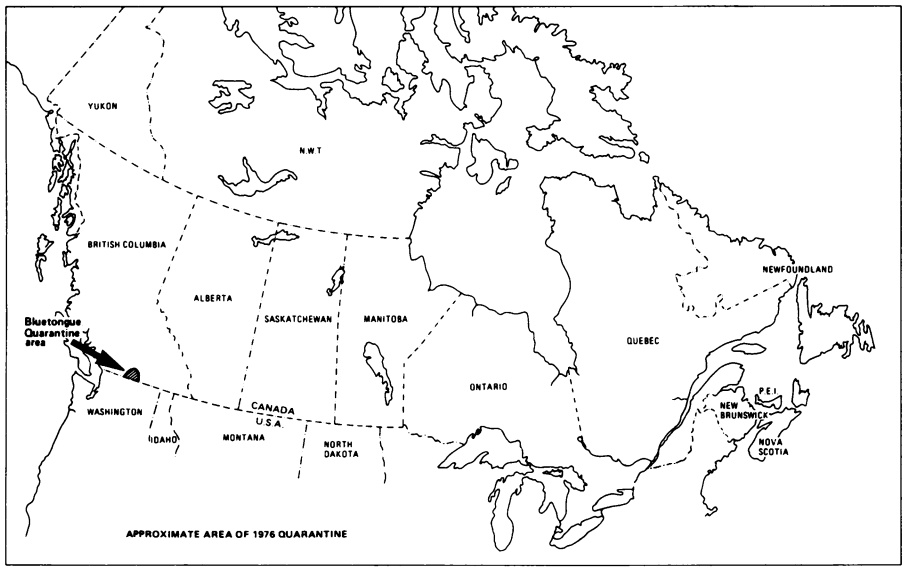

Fig. 1. Approximate area of 1976 quarantine.

Canada was declared infected by the Agriculture Canada Health of Animals Branch and a quarantine of approximately 1 200 square miles was placed on an area of British Columbia including the Okanagan Valley (Fig. 1). There were no clinical signs observed. Attempts were made to bleed all cattle within the area and reactors were slaughtered.

The present report briefly describes the monitoring for serological reactors amongst cattle and some other ruminants, virus isolation attempts from these and pools of *Culicoides* flies and subsequent serological monitoring in Canada.

MATERIALS AND METHODS

SPECIMEN COLLECTION AND PROCESSING

Bovine tissues from several animals were collected during the 1976 slaughter.

Bovine Tissues — Hundred mL quantities of whole blood containing the anticoagulant EDTA were collected from each of 154 seropositive cattle and shipped on ice to the laboratory. Fetal tissues were collected from 46 seropositive dams at slaughter. These comprised visceral organs, usually including spleen, lung, liver and lymph nodes. Spleen samples were taken from 24 calves accompanying seropositive dams. The fetal tissues and spleen samples were shipped frozen (dry ice) in pieces of several grams in sealed containers and held at -20°C until processed.

The blood samples were processed by washing the cellular components three times in diluent,[1] resuspending to 20% of their original volume, and sonically disrupting the cells in an ice bath using two five second treatments with a Sonifer[2] cell disrupter. They were then inoculated into sheep (one sample per sheep, half intravenously (iv) and half subcutaneously), 11-day old chicken embryos (iv) and L-929[3] mouse fibroblast cell cultures (9). The sheep were subjected to "blood autografting" on postinoculation days (pid) 5, 6, 7, and 8 (9). Blood was collected for passage on pid 0,15 and all days of temperature rise, and for serology on pid 0,21,35, and 50.

The fetal tissue and calf spleens were emulsified in diluent, sonically treated and inoculated into sheep, embryonating eggs and L-929 cell cultures.

The protocol for virus isolation is given in Fig. 2.

Culicoides Flies — In the late summer and autumn of 1976, a total of 6 000 to 7 000 flies of various genera (6) of *Colicoides* species were trapped, snap frozen on

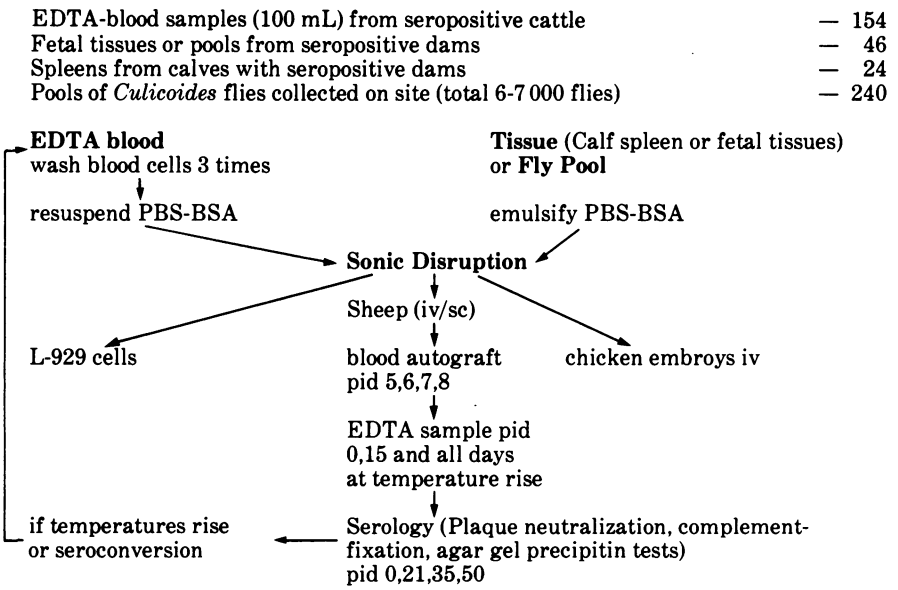

Fig. 2. Samples and processing methods for bluetongue virus isolation attempts — 1976.

[1]Phosphate buffered saline with 0.5% bovine serum albumin added.
[2]Heat Systems Ultrasonics Inc., 38 East Mall, Plainview, New York.
[3]American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland.

dry ice, and processed in 240 pools as the bovine tissues (above). These were inoculated into sheep, eggs, and cell cultures (Fig. 2) (2,3,8,9).

A similar collection of *Culicoides* flies was made in 1977, comprising approximately 500 flies in 45 pools. These were processed for virus content as described above.

A summation of the specimens processed is given in Fig. 2.

SEROLOGY

The serum samples were tested by modified direct complement fixation test using mouse brain antigen as described earlier (10).

In 1976, 37 338 cattle from 151 herds in the quarantine area were tested as were 730 sheep, 328 goats and 63 mule deer. In British Columbia outside the quarantine area, 4 675 cattle were tested. From areas near the U.S. border in Ontario, Manitoba, Saskatchewan and Alberta, an additional 18 034 cattle were tested.

In 1977, a total of 53 018 cattle were tested from across Canada, including 35 815 from British Columbia.

In 1978, a total of 22 947 cattle serums were collected from areas within a 20 mile radius of artificial insemination centers in Ontario, Quebec, Alberta and British Columbia and from the Okanagan Valley in the 1976 quarantine area.

In 1979, a total of 5 102 cattle serums were collected from the Maritime provinces, Ontario, Alberta, and British Columbia.

The number and origin of the bovine serums are given in Table I.

TABLE I. Bluetongue Complement Fixation Test Reactors Amongst Canadian Cattle

Year	Location	No. Reactors/No. Tested	% Positive
1976	British Columbia (quarantine area)	1418/37338	3.80
	British Columbia (around quarantine area)	68/4675	1.45
	Alberta	0/5980	0
	Saskatchewan	0/1004	0
	Manitoba	0/3659	0
	Ontario	0/7391	0
	Total:	1486/60047	2.47
1977	British Columbia	17/35815[a]	0.05
	Alberta	2/2248[b]	0.09
	Saskatchewan	0/1579	0
	Manitoba	0/2126	0
	Ontario	0/7449	0
	Québec	0/3442	0
	Maritimes	0/359	0
	Total:	19/53018	0.04
1978	British Columbia	3/10,211[a]	0.03
	Alberta	0/421	0
	Québec	0/706	0
	Ontario	0/11,609	0
	Total:	3/22947	0.01
1979	British Columbia	2/1604[a]	0.12
	Alberta	0/114	0
	Ontario	0/409	0
	Maritimes	0/2975	0
	Total:	2/5102	0.04

[a]Inside quarantine area
[b]U.S.A. imports

RESULTS

No virus isolation was made from the samples by any of the systems used and there was no seroconversion by any of the test sheep (Fig. 2).

In the four years of testing from 1976 to 1979, a total of 1 510 seropositive cattle were identified from a total of 141 114 tests. All but 24 were from the 1976 testing. All were from the quarantine area or immediately adjacent to it in British Columbia (Table I), except for two U.S. imports identified in Alberta in 1977. From the 1976 sampling in the quarantine area, 15 of the 730 sheep, seven of the 328 goats and four of the 63 mule deer were seropositive.

DISCUSSION

Several authors have reported on the isolation of field isolates of BTV by one or more of the methods used in this study (1-5,7-9). Because of the amount of blood processed, the number of animals tested, the number of *Culicoides* sampled and the multiplicity of test systems used, it seems certain that BTV was not active in the areas in question. The results of the serological monitoring, notably the dramatic decrease in the prevalence of seropositive animals, support this hypothesis. It appears likely then, that the small quarantined part of British Columbia known to be ecologically a rather unique part of Canada, represents an area in which BTV is usually not active but into which it spread, probably from known endemic areas to the south. Such waves of BTV spread into "clean" areas are rather typical of BTV epidemiolоly and failure of the virus to become endemic is probably related to effects of climatic and other unknown environmental factors on vector populations. Bluetongue virus apparently did not become established in the Okanagan Valley area of Canada after an incursion prior to 1976.

It is noteworthy that no serological reactors were identified during the 1976 to 1979 testing of animals across Canada from British Columbia to the Maritime provinces supporting the assumption that Canada has been essentially BTV free.

Bluetongue virus infection has been suspected of being transmitted congenitally, with or without the development of substantial

antibody titers (5). This of course represents a markedly different mode of transmission for the classical arbovirus spread normally assumed for BTV. The question of the significance of the nonarbovirus type of infection to the natural survival of BTV has not been answered, however. Since BTV often circulates in the absence of observed clinical signs, as it apparently did in the present case, and since the congenital mode of transmission may result in low or even undetectable antibody levels, it is very difficult to determine the frequency with which congenital transmission may occur naturally. However, assuming that some animals would eventually develop antibody even if the congenital mode of transmission were operative, there is no evidence for a nonanthropod borne type of transmission in the present study area. Since most of these cattle were range animals for which natural service was used, ample opportunity should have occurred for congenital transmission. This combined with the absence of virus detection in fetuses of seropositive dams or in calves from seropositive dams suggests that BTV does not always establish nonarthropod-borne transmission. Since Canadian cattle appear to rarely show evidence of infection (i.e. seroconversion), an observation which would be unexpected if congenital transmission were a long term and important part of BTV epidemiology, it must be concluded that BTV usually behaves like a classical arbovirus.

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REFERENCES

1. **BRECKON, R.D., A.J. LUEDKE** and **T.E. WALTON.** Bluetongue virus in bovine semen: viral isolation. Am. J. vet. Res. 41:439-442. 1980.
2. **FOSTER, N.M., A.J. LUEDKE** and **H.E. METCALF.** Bluetongue in sheep and cattle: Efficacy of embryonating chicken eggs in viral isolations. Am. J. vet. Res. 33:77-82. 1972.
3. **GOLDSMIT, L.** and **E. BARZILAI.** An improved method for the isolation and identification of bluetongue virus by intravenous inoculation of embryonating chicken eggs. J. comp. Path. 78: 477-487. 1968.
4. **LUEDKE, A.J., M.M. JOCHIM** and **R.H. JONES.** Bluetongue in cattle: Viremia. Am. J. vet. Res. 30: 511-516. 1969.
5. **LUEDKE, A.J., R.H. JONES** and **T.E. WALTON.** Overwintering mechanism for bluetongue virus: Biological recovery of latent virus from a bovine by bites of Culicoides variipennis. Am. J. trop. Med. Hyg. 26: 313-325. 1977.
6. **McMULLEN, R.D.** Culicoides (Diptera: Ceratopogonidae) of the south Okanagan area of British Columbia. Can. Entomologist 110: 1053-1057. 1978.
7. **OSBURN, B.I., B. McGOWAN, B. HERON, E. LOOMIS, R. BUSHNELL, J. STOTT** and **W. UTTERBACK.** Epizootiologic study of bluetongue: virologic and serologic results. Am. J. vet. Res. 42:884-887. 1981.
8. **THOMAS, F.C., N. WILLIS** and **G. RUCKERBAUER.** Identification of viruses involved in the 1971 outbreak of hemorrhagic disease in southeastern United States white-tailed deer. J. Wildl. Dis. 10: 187-189. 1974.
9. **THOMAS, F.C., N.G. WILLIS, G.M. RUCKERBAUER, A. GIRARD** and **P. BOULANGER.** A comparison of methods for monitoring viremia in a bluetongue virus infected bovine. Ann. Proc. Am. Ass. Vet. Lab. Diag. 18: 175-186. 1975.
10. **THOMAS, F.C., A. GIRARD, P. BOULANGER** and **G. RUCKERBAUER.** A comparison of some serological tests for bluetongue virus infection. Can. J. comp. Med. 40:291-297. 1976.