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APPLICATION OF A COMPETITIVE ELISA FOR THE DETECTION OF BLUETONGUE VIRUS ANTIBODIES IN LLAMAS AND WILD RUMINANTS

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ABSTRACT: A competitive enzyme-linked immunosorbent assay (C-ELISA), using a group-specific monoclonal antibody against bluetongue virus (BTV), was applied to detect anti-BTV antibodies in serum samples from two llamas (*Llama glama*) experimentally infected with BTV serotype 10. Antibodies were detected in both llamas by 1 wk or 2 wk post-infection. Antibodies to BTV increased exponentially during the first 4 wk in both llamas and stabilized at an elevated level during the remaining 5-wk-period of the experiment. We evaluated the C-ELISA for 1,442 field sera from bluetongue-free areas, collected from 398 llamas in New Zealand as well as 451 elk (*Cervus elaphus canadensis*), 323 bison (*Bison bison*) and 270 reindeer (*Rangifer tarandus tarandus*) in Canada. Based on the frequency distribution of the C-ELISA values, we propose that the current negative cut-off value of 50% inhibition established for bovine field sera also can be applied to the sera from these wild ruminants. The C-ELISA values for other wild ruminant field sera collected in bluetongue-free areas of Canada from 98 native caribou (*Rangifer tarandus caribou*), 32 white-tailed deer (*Odocoileus virginianus*), 14 moose (*Alces alces*), and nine musk-oxen (*Ovibos maschatus*) and 15 yak (*Bos grunniens*) also were less than 50%, with the exception of three caribou samples. Based on our results, we propose that the C-ELISA be used as a rapid and specific test for serodiagnosis of BTV infection in llamas and possibly other wild ruminants.
Key words: Bluetongue, competitive ELISA, llamas, wild ruminants.

INTRODUCTION

Bluetongue (BT) is an infectious, non-contagious, viral disease of domestic and wild ruminants (Erasmus, 1985). At least 24 serotypes of BT virus (BTV), an arthropod-borne orbivirus of the Reoviridae family (Spence et al., 1984), have been identified world-wide (Gorman, 1990). Clinically, BT can occur as an acute, subacute, mild or inapparent disease. In domestic sheep and white-tailed deer (*Odocoileus virginianus*) clinical signs develop rapidly, with a short virus incubation and viremic period. Cattle, goats, elk (*Cervus elaphus canadensis*) and other wild ruminants develop subacute or inapparent infections and are considered to have a longer viremia (Sohn and Yuill, 1991).

Diagnosis of BTV infection, often not clinically noticeable in cattle, is based on group-specific reactive serological assays and serotype-specific neutralization tests (Pearson et al., 1985). The agar gel immunodiffusion (AGID) test (Pearson et al., 1985) and the competitive enzyme-linked immunosorbent assay (C-ELISA) (Afshar et al., 1987) are the most widely used tests for the detection of group-specific anti-BTV antibodies.

Based on recent reports on validation of monoclonal antibody (MAb)-based C-ELISA's, this assay is superior to the AGID test (Afshar et al., 1989; Reddington et al., 1991).

Since the development of the C-ELISA, we have used the assay for testing a limited number of sera collected from wild ruminants, especially those involved in the 1987 to 1988 epizootic of BT in the Okanagan Valley of British Columbia, Canada (Dulac et al., 1992). We arbitrarily have used the negative cut-off value established for cattle and sheep sera (Afshar et al., 1989) to diagnose serological reactivity in sera from other animal species. However, because of the recent trends in livestock trade and international movement of wild ruminants, our objective was to establish C-ELISA cut-off values for selected species by testing a large number of samples. Fur-

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TABLE 1. Bluetongue Competitive ELISA (C-ELISA) results of 1,442 serum samples collected from wild ruminants in Bluetongue-free areas.

C-ELISA (% inhibition category)	Number of serum samples			
	Elk[a]	Llama[b]	Bison[a]	Reindeer[a]
0–10	227	251	54	136
11–20	179	129	89	105
21–30	28	18	82	21
31–40	12	0	42	2
41–50	4	0	33	5
51–60	0	0	14	0
61–70	1	0	6	1
71–80	0	0	2	0
81–90	0	0	0	0
91–100	0	0	1	0
Total	451	398	323	270

[a] Samples collected in Canada.
[b] Samples collected in New Zealand.

thermore, we describe the performance of the C-ELISA in measuring the humoral antibody response in llamas (*Llama glama*) experimentally inoculated with BTV serotype 10.

MATERIAL AND METHODS

In January 1993, two healthy adult male llamas obtained from Llamas of Michigan (Caledonia, Michigan, USA) were housed in an insect-proof animal isolation room of a biocontainment facility of the Animal Diseases Research Institute, Nepean, Ontario, Canada. Each llama was inoculated by intravenous (10.0 ml), subcutaneous (1.0 ml) and intradermal (1.0 ml) routes with a suckling mouse brain stock of BTV serotype 10, containing approximately $10^{8.4}$/ml plaque forming units of the virus. Serum samples were obtained prior to inoculation and on weekly intervals post inoculation for 9 wk. The llamas were housed in isolation facilities and were cared and handled according to the guidelines of the Canadian Council on Animal Care.

Sixteen hundred and one sera from wild ruminants were tested by the C-ELISA (Afshar et al., 1989). These sera were from 398 llama collected in 1992 in New Zealand, a BT-free country (Doyle, 1992); 451 elk (*Cervus elaphus canadensis*), 323 bison (*Bison bison*) and 270 reindeer (*Rangifer tarandus tarandus*), collected between 1990 and 1993 from eastern (45° to 46°N, 70° to 75°W) and midwestern (50° to 60°N, 104° to 115°W) regions in Canada, a BT-free country (Dulac et al., 1992). These samples were tested to establish the negative cut-off values for each species. Other wild ruminant serum samples collected between 1988 and 1993 from 98 caribou (*Rangifer tarandus caribou*), 32 white-tailed deer (*Odocoileus virginianus*), 14 moose (*Alces alces*), and nine musk-oxen (*Ovibos maschatus*) native to Canada, and 15 yak (*Bos grunniens*) raised in Canada, also were tested. Caribou samples were collected from Labrador and the Northwest Territories. The other wild ruminant samples were collected from British Columbia and Alberta, Canada. All sera were transported on ice and stored at −20 C until tested.

Serum samples from experimentally infected llamas were tested for neutralizing antibodies to BTV serotype 10 according to the microtiter serum neutralization (MTSN) test described by Pearson et al. (1985).

RESULTS

Both llamas inoculated with BTV serotype 10 remained healthy throughout the period of this study and had no clinical signs suggestive of bluetongue. However, both animals developed antibodies to BTV infection. Bluetongue virus antibodies were detected by the C-ELISA test, at approximately 70% inhibition value as early as 1 wk post-infection (WPI) in one llama and 2 WPI in the other. The C-ELISA group-specific antibodies to BTV increased exponentially during the first four weeks in both llamas before becoming relatively stable at elevated levels of 90% inhibition values during the remaining 5 wk of the experiment. Neutralizing antibodies first were detected by the MTSN test, at 1:20 serum dilution, between 2 and 3 WPI in the llamas and then elevated to higher levels of 1:80 to 1:160 during the remaining period of the experiment.

All sera with the exception of one elk, one reindeer and 23 bison serum samples, had C-ELISA values of less than 50% inhibition (Table 1).

The C-ELISA testing of 168 sera collected from caribou, white-tailed deer, moose and musk-oxen, all native to Canada, and yak raised in Canada resulted in inhibition values of less than 50% with the exception of three samples from caribou.

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DISCUSSION

Based on our findings, we believe that the BT C-ELISA has potential value for serodiagnosis of bluetongue in llamas and possibly other wild ruminants. The antibody response in llamas experimentally inoculated with BTV serotype 10 was similar to those reported for cattle and sheep (Afshar et al., 1987). Anti-BTV antibody activity first was evident in the sera of the llamas 1 and 2 wk after infection by the C-ELISA, whereas serum neutralizing antibodies to BTV-10 were not detected in both llamas until 2 and 3 WPI. Like cattle (Afshar et al., 1987), no clinical changes were observed in llamas experimentally infected with BTV-10. Fowler (1989), however, reported a case of abortion in a llama associated with BTV infection, as evident by a four-fold increase in antibody titer. In our experimental study, we did not attempt to recover BTV from blood samples, although the humoral antibody response is evidence for viral replication (Afshar, 1994). A comprehensive experimental study would be required to demonstrate the length of viremia in llamas in order to substantiate their role as a reservoir of BTV in enzootic areas such as South America.

We used a large sample size of field sera from elk (n = 451), llamas (n = 398), bison (n = 323), and reindeer (n = 270) in BT-free areas of New Zealand and Canada to establish the C-ELISA negative cut-off value for each species. Based on the frequency distribution of the C-ELISA values, we selected an arbitrary cut-off value of 50% inhibition, which resulted in the assay specificity of 99.8%, 100%, 92.9% and 99.6%, respectively. Twenty-five samples from bison (n = 23), elk (n = 1) and reindeer (n = 1) had inhibition values above, but close to the 50% negative threshold and were classified as false positives. Similar to our previous report for cattle sera (Afshar et al., 1993), further testing of these false positive sera in the "tandem" blocking ELISA format and another C-ELISA, using a different MAb to BTV, eliminated the false reactions in all the sera with the exception of two serum samples from bison (A. Afshar, unpubl.). The two bison sera were toxic and unfit for serum neutralization assay. Unfortunately, the few known positive sera available for this study precluded an accurate definition of the analytical and diagnostic sensitivity, including prevalence of false negative reactions, of the C-ELISA test when applied to wild ruminants exposed to BTV. Furthermore, the testing of such sera would establish the competitiveness of their elicited immunoglobulins with the MAb for binding to specific epitope of BTV antigen used in the C-ELISA. Based on our results with llamas, we believe that antibodies to VP7, a major core protein of BTV serotype 10, are produced in great numbers among camelids, following BTV infection. However, we recognise that additional work must be done with larger numbers of known positive samples before a firm conclusion on the diagnostic performance of the C-ELISA can be drawn.

Preliminary results on the testing of a limited number of sera from other wild caribou, white-tailed deer, moose and musk-oxen, native to Canada, and yak, raised in Canada, provided evidence that the cut-off value of 50% inhibition for cattle and sheep also may be suitable for classification of other wild ruminant sera. We currently are obtaining additional serum samples from these species for C-ELISA testing in order to verify the selected cut-off value for other wild ruminants.

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