Improvements in the Modified Direct Complement Fixation Test and its Application in the Detection of Bluetongue Antibodies in Cattle and Sheep Sera

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ABSTRACT

In this study, improvements were made in the technique and the preparation of the antigen. It was possible to perform three extractions and elutions resulting in a soluble reactive preparation from each batch of infected mouse brain. This led to an appreciable increase in the yield of highly reactive antigen. The presence of bluetongue antibodies was not detected in 13,210 sheep sera. Of the 13,486 bovine sera tested, only three questionable reactions were obtained. It was possible to determine that two of these animals were imported. Various isolation methods, including transmission trials to susceptible sheep followed by serological tests on the sheep sera, failed to confirm the infection in the three reactors.

RÉSUMÉ

Au cours de cette étude, les auteurs ont amélioré la technique et la préparation de l'antigène. Ils réussirent à effectuer trois extractions de chacun des lots de cerveaux de souris infectées; cela se traduisit par une augmentation appréciable de la récolte d'un antigène soluble de haute qualité. Ils ne décelèrent pas d'anticorps de la fièvre catarrhale du mouton, dans les 13,210 échantillons de sérum ovin qu'ils éprouvèrent à cette fin. Ils n'obtinrent que trois réactions douteuses, lors de l'épreuve de 13,486 échantillons de sérum bovin. Deux de ces trois animaux représentaient des sujets importés. L'emploi de diverses méthodes d'isolation du virus, y compris des essais de transmission à des moutons susceptibles et l'examen ultérieur de leur sérum par des méthodes sérologiques, ne permit pas de confirmer la présence de l'infection chez les trois bovins douteux.

Due to the spread of bluetongue (BT) from its country of origin to other parts of the world and its appearance in the United States in 1953 (3, 4), it was thought desirable to report the health status of Canadian livestock. The modified direct

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complement fixation test (MDCF), developed previously in our laboratory (1, 5) for the study of vesicular stomatitis, (2), has been applied for the diagnosis of BT. In recent years, this method was used for the certification of animals for export and import.

The BT viral antigen consisted of acetone-ether extracts of California Type-10 virus-infected mouse brain. The brains of infected suckling mice were collected aseptically and the antigen was extracted by homogenizing one volume of brain tissue with 20 volumes of cold acetone. The tissue suspension was agitated for two hours on a mechanical shaker held in a refrigerator at 4 to 9°C. The suspension was then centrifuged at 1200 x g for ten minutes and the supernatant fluid was discarded. The tissue extraction was repeated once with acetone, then a 50:50 mixture of acetone-anhydrous ether and twice with anhydrous ether. After the last extraction and centrifugation, the supernatant fluid was decanted and the residual ether was avaporated from the tissue by manual agitation with a glass rod. The BT antigen was eluted from the dry powder by adding 2 ml of physiological saline for each gram of original brain tissue. This suspension was transferred to a stainless steel container and sonicated for six minutes with a Branson J-17A sonicator set at 20,000 cycles per second. Overheating was avoided by placing the container in an ice bath and interrupting the sonication process for one minute after each minute of sonication. The tissue suspension was then frozen for two to three days at –70°C. Subsequently, the sonicated suspension was centrifuged at 13,000 x g for 20 minutes in a refrigerated centrifuge. The supernatant fluid was kept as the antigen. A second and third elution was performed by adding respectively 1 ml and 0.5 ml of physiological saline for each gram of original brain tissue. The sonication, freezing and centrifugation were repeated. The antigen was stored lyophilized. For a normal antigen control, uninoculated mouse brains were extracted as above.

It was possible to perform three elutions and obtain reactive antigen from the infected brain tissue suspension. The three eluates from each batch of tissue were tested individually and again after being pooled and lyophilized for stock antigen. The various batches of stock antigen had end-point titers varying from 1:32 to 1:64 after pooling. Consequently, the four units of antigen utilized in the test were obtained by diluting the stock suspension between 1:8 and 1:16.

In addition to the improvement in the preparation of the antigen, it was also found advantageous to lyophilize the serum modifying factor and the guinea-pig complement which could then be stored for a long period without loss of potency. During the incubation of the test at 4-9°C, a deterioration of the complement takes place. In the direct CF test, this is compensated for by a correction factor of 1.5. However, in the MDCF test for BT it was found that a greater deterioration of complement takes place in the presence of the bovine modifying factor. Consequently, it was necessary to use a correction factor of 1.8 instead of 1.5. This resulted in the addition to the test of 5.4 fifty percent hemolytic units of complement in order to obtain three units at the end of the overnight incubation period.

The MDCF test was used to test the sera of 12,978 cattle across Canada from April 1970 through January 1974 (Table I). Out of these field bovine sera, only three from one premise, gave questionable reactions. It was possible to determine that two of these three animals were imported. The third animal was obtained for experimental studies and repeated attempts to isolate the viral agent by various methods including transmission trials in susceptible sheep, with serological monitoring, were unsuccessful. A further 508 animals from artificial insemination units (AI) located in the provinces of British Columbia, Alberta, Saskatchewan, Ontario and New Brunswick were also tested with negative results. A total of 11,433 sheep were used in trans-

TABLE I. Results of the Modified Direct Complement Fixation Test on Canadian Cattle Sera Excluding Those from Artificial Insemination Units

Years	Number of Tests	Reactions
April-70 to March-71	192	0
April-71 to March-72	2,829	0
April-72 to March-73	4,660	3 Questionable
April-73 to Jan.-74	5,297	0
Total	12,978	3 Questionable

mission trials using blood and semen of the animals in AI units. These sheep were tested before inoculation of blood or semen from the bulls and between 48 and 53 days later. An additional 1,777 sheep were pretested but not needed in the transmission experiment. Consequently no bluetongue reaction was detected in a total of 26,643 MDCF tests performed on 13,210 sheep.

In a number of courtesy tests performed for other countries on both bovine and ovine sera, we have observed strong reactions which persisted at high levels for over a year and as long as three years in one instance. These results differ from those observed in experimental transmissions performed with animals in isolation (5) where significant antibodies were detectable in some cases for only a few months. Consequently, it seems logical to assume that, if any of the animals tested in the present study had been infected in the field with BT virus, antibodies would have been detected in high levels in their sera by the MDCF test.

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