

THE USE OF TISSUE CULTURE PROPAGATED BLUETONGUE
VIRUS FOR COMPLEMENT FIXATION STUDIES ON
SHEEP SERA.

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While the potential value of a complement fixation test for studies on bluetongue in sheep has long been apparent, the lack of a satisfactory antigen appeared to be the main obstacle in the development of such a test. Antigens prepared from bluetongue-infected chicken embryo and mouse brain have been used with some success to detect antibodies in bluetongue-immune mouse serum (Van den Ende, Linder & Kaschula, 1954). However, when tested against bluetongue hyperimmune or convalescent sheep serum, these preparations gave very inconsistent results. (McKercher, McGowan and Saito, 1954).

The finding that bluetongue virus can be propagated readily in lamb kidney cell cultures (Haig, McKercher and Alexander, 1956) suggested that such cultures might provide a satisfactory complement-fixing antigen for the detection of bluetongue antibodies in the serum of sheep. Accordingly, studies along this line were undertaken and are reported herein.

MATERIALS AND METHODS.

Strains of Virus.

The origin of the Cyprus and Theiler strains has been given by Neitz (1948); that of the California 8 strain by McKercher *et al.* (1954).

The Estantia, Vlak, Bloukop and Ermelo strains were isolated from previously vaccinated sheep in the Eastern Transvaal region of the Union of South Africa.

Sera.

Immune sera were obtained from sheep two months after infection with unmodified strains of bluetongue virus. The sera were stored at minus 20° C. and inactivated immediately before use at 56° C. for thirty minutes.

Normal sera were obtained from sheep in a group subsequently shown to be bluetongue susceptible.

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Antigens.

The Cyprus and Estantia strains of bluetongue were propagated in lamb kidney cell cultures by the method described by Haig *et al.* (1956).

For this work virus from the 4th serial tissue culture passage was employed.

At the time of inoculation the cell culture medium was replaced with serum free medium. When cytopathogenesis was complete, the cultures were stored in a refrigerator for periods up to seven days. They were centrifuged lightly before use and the undiluted supernatant fluid was used for these tests.

Diluent.

Veronal buffer (Brooksby, 1952) was used as the diluent in all tests.

Complement.

Guinea-pig serum from adult males provided the complement. The sera were pooled and held at 4° C. overnight, in the presence of washed sheep red cells, to remove natural anti-sheep red cell haemolysin. It was then stored under dry ice refrigeration in sealed glass ampoules.

Anti-sheep cell haemolysin.

Anti-sheep red cell haemolysin prepared by the intravenous injection of rabbits with fifty per cent suspension of washed sheep cells was obtained from supplies available for routine tests at the Institute. It had been inactivated at 60° C. for thirty minutes and stored in the frozen state.

Sheep red blood cells.

Blood from bluetongue susceptible sheep was drawn into an equal volume of Alsevers fluid and stored at 4° C. The cells were washed three times in saline immediately before use and made up to a 2 per cent solution in veronal buffer.

Test Procedure.

Each reagent in the test was used in a volume of 0·2 c.c. Serial two-fold dilutions of the sera were prepared. Antigen and two full units of complement (titrated in the presence of antigen) were added. The mixture was incubated in a water bath of 37° C. for ninety minutes.

To each tube was added 0·4 c.c. of a 2 per cent sheep red cell suspension, sensitised with an equal volume of haemolysin. The haemolysin was used at optimal dilution. The tubes were re-incubated for 30 minutes, after which the readings were made.

Adequate controls were included in the tests.

EXPERIMENTAL.

The results of the tests are given in Table 1.

TABLE 1.

Complement Fixation Test with Tissue Culture Antigens and Sheep Sera.

Antigen.	Sera.	SERUM DILUTIONS.			
		1/8.	1/16.	1/32.	1/64.
Cyprus.....	Cyprus Immune.....	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	—	*
	Estantia Immune.....	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷
	Theiler Immune.....	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷
	Vlak Immune.....	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	—
	California 8 Immune....	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	—	—
	Normal control.....	—	—	—	—
Estantia.....	Cyprus Immune.....	÷ ÷ ÷ ÷	÷ ÷	—	—
	Estantia Immune.....	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	÷ ÷ ÷
	Theiler Immune.....	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	÷ ÷ ÷
	Vlak Immune.....	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	—
	California 8 Immune....	÷ ÷ ÷ ÷	÷ ÷ ÷ ÷	—	—
	Normal Control.....	—	—	—	—
Uninoculated Kidney cell Culture	Cyprus Immune.....	—	—	—	—
	Estantia Immune.....	—	—	—	—
	Theiler Immune.....	—	—	—	—
	Vlak Immune.....	—	—	—	—
	California 8 Immune....	—	—	—	—
	Normal control.....	—	—	—	—

* — = No haemolysis.

÷ ÷ ÷ ÷ = Complete haemolysis.

Results.

From the Table it is seen that with either of the two tissue culture propagated strains of virus used as antigen a concentration of serum not greater than 1/16 was required to demonstrate the fixation of complement. With serum from sheep immune to two strains of virus (Estantia and Theiler) an end point was not reached at a dilution of 1/64. The complement-fixing titre of each of the sera investigated was practically identical for either of the virus antigens used and therefore appears to be correlated with the serum and not with the antigenic structure of the virus strain. Since the two strains used as antigen are known to be widely separated immunologically when tested on sheep (Neitz, 1948) it may be concluded that the test described is not strain specific.

DISCUSSION.

The complement-fixing antigen described in this preliminary publication is easily and rapidly prepared in large quantities. Since the culture fluid contains no serum the expectation that it would be devoid of anti-complementary activity was fulfilled.

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The value of the test in studies on the immunology or epizootiology of bluetongue lies chiefly in the fact that it has a wide spectrum and therefore that it can be used to detect the presence of antibodies in the serum of sheep irrespective of the antigenic structure of the virus strain involved. Thus it can be applied to a survey of the areas infected in the field and particularly for diagnostic purposes in those cases where the symptoms shown by affected animals are so mild and indefinite that an undisputed clinical diagnosis is not possible. Used in conjunction with the strain specific *in vitro* neutralization of cytopathogenicity of egg adapted virus there has been made available a technique which should prove to be of great value.

SUMMARY.

The development of a complement-fixation test possessing broad specificity, in which tissue culture propagated virus is used as antigen, for studies on bluetongue in sheep is described.

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This article is published with the approval of the Director of Veterinary Services, Southern Rhodesia, and the Dean, Faculty of Veterinary Medicine, University of California, Davis, California, United States of America.