

Isolation of bluetongue virus from sheep in Rajasthan State, India

G. PRASAD, A.K. GARG, MINAKSHI, N.K. KAKKER and R.N. SRIVASTAVA *

Summary: A cytopathic agent was isolated in a baby hamster kidney (BHK)-21 cell-line from blood samples of cross-bred sheep showing typical bluetongue symptoms at Avikanagar in Rajasthan State, India. The cytopathic agent was identified as a bluetongue virus (BTV) by immunofluorescence, the immunoperoxidase test and electron microscopy of BHK-21 cells infected with the new isolate. The new isolate was typed as BTV serotype 1.

KEYWORDS: Bluetongue virus - Electron microscopy - Immunofluorescence - Immunoperoxidase test - India - Sheep.

INTRODUCTION

Bluetongue virus (BTV) is a double-stranded RNA virus belonging to the genus Orbivirus of the family Reoviridae, and is transmitted by *Culicoides* spp. BTV can infect several species of domestic and wild ruminants, but sheep are the most susceptible species. Although BTV infection is endemic in India (7), there has been no report to date of BTV isolation from Rajasthan State, India. The authors report the isolation of BTV from a flock of sheep kept at the Central Sheep and Wool Research Institute at Avikanagar in Rajasthan State.

MATERIALS AND METHODS

Isolation of the virus

Blood samples from sheep showing high temperature and other clinical symptoms of bluetongue were collected in heparin (10 international units/ml). Samples were packed in ice and transported to the laboratory by courier. The samples were pooled and processed for virus isolation in a baby hamster kidney (BHK)-21 cell-line, following the method described by Jeggo *et al.* (6).

Identification of the new isolate

A cytopathic agent was isolated from the pooled blood samples. The agent was identified by immunofluorescence, the immunoperoxidase test and electron microscopy.

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For immunofluorescence, BHK-21 cell monolayers were grown on coverslips using Leighton tubes. The cultures were infected with the cytopathic agent. At 24 h and 48 h post-incubation (p.i.), both infected and non-infected coverslip cultures were fixed with a chilled mixture of acetone (80%) and methanol (20%), and subjected to the indirect fluorescent antibody test, in accordance with the procedure described by Whetter *et al.* (8).

For indirect immunoperoxidase staining, the BHK-21 monolayers were grown on coverslips and infected with the new isolate. The cultures were fixed in chilled acetone at 24 h and 48 h p.i. After fixing, the cultures were placed in a phosphate buffer solution (PBS) bath for 5 min. To remove endogenous peroxidase, the cultures were quenched with quenching solution (absolute methanol plus 3% hydrogen peroxide) for 20 min. at 25°C. The slides were then washed thoroughly with PBS and processed further for immunoperoxidase staining as previously described (1).

The new isolate showing cytopathic effect (CPE) in BHK-21 cells was subjected to transmission electron microscopy according to the method described by David *et al.* (2). The new isolate was sent to the Institute for Animal Health, Pirbright Laboratory, United Kingdom for serotyping.

RESULTS AND DISCUSSION

The BHK-21 cells inoculated with the pooled blood samples showed rounding at 72-96 h p.i. in the first passage. In subsequent passages, the extent of CPE increased, and at the sixth passage almost 90% of the BHK-21 cells exhibited CPE and became detached from the surface of the culture bottles at 48 h p.i. The persistence of CPE in subsequent passages – even after passage through a cellulose acetate filter (0.45 µm) – suggested that the cytopathic agent was possibly a viral agent.

Indirect immunofluorescence and the immunoperoxidase test used to identify the cytopathic agent revealed the presence of BTV-specific fluorescence and brown colour reaction, respectively, in the cytoplasm of the infected BHK-21 cells. The results of both tests suggested that the cytopathic agent was BTV.

Observation by electron microscopy of infected BHK-21 cells indicated the presence of numerous viral particles with characteristic BTV morphology. The presence of tubular structures in the cytoplasm of the infected BHK-21 cells, which have been ascribed to the accumulation of BTV non-structural protein (NS1), also supported the conclusion that the new isolate was BTV. The tubular structures in BTV-infected cells have been described previously (3, 4).

This is the first report of the isolation of BTV from Rajasthan, an area which has a very large sheep population. The isolation of BTV serotype 1 has been reported from Haryana, a state adjoining Rajasthan (5). The new isolate was typed as BTV serotype 1 by P.C. Mertens at the Pirbright Laboratory.

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ISOLEMENT DU VIRUS DE LA FIÈVRE CATARRHALE DU MOUTON CHEZ LES OVINS DANS L'ÉTAT DU RAJASTHAN, EN INDE. – G. Prasad, A.K. Garg, Minakshi, N.K. Kakker et R.N. Srivastava.

Résumé : A Avikanagar, dans l'Etat du Rajasthan, en Inde, un agent cytopathogène a été isolé sur la lignée 21 de cellules rénales de hamster nouveau-né (baby hamster kidney : BHK-21). Cet agent a été isolé d'un prélèvement sanguin effectué sur des ovins croisés qui présentaient tous les symptômes de la fièvre catarrhale du mouton. L'agent cytopathogène a été reconnu comme étant le virus de la fièvre catarrhale par les techniques de l'immunofluorescence et de l'immuno-peroxydase ainsi que par l'observation au microscope électronique de cellules BHK-21 infectées, et lors du typage il a été identifié comme étant le sérotype 1 de ce virus.

MOTS-CLÉS : Immunofluorescence – Inde – Microscopie électronique – Ovins – Technique de l'immuno-peroxydase – Virus de la fièvre catarrhale du mouton.

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AISLAMIENTO DEL VIRUS DE LA LENGUA AZUL EN OVINOS DEL ESTADO DE RAJASTÁN, INDIA. – G. Prasad, A.K. Garg, Minakshi, N.K. Kakker y R.N. Srivastava.

Resumen: En Avikanagar, en el estado de Rajastán, India, se aisló un agente citopatógeno en la línea 21 de células renales de hámster recién nacido (baby hamster kidney: BHK-21), a partir de muestras de sangre tomadas de ovinos cruzados que presentaban todos los síntomas de la lengua azul. El agente citopatógeno fue reconocido como virus de la lengua azul mediante las técnicas de inmunofluorescencia e inmunoperoxidasa, así como también mediante la observación en microscopio electrónico de células BHK-21 infectadas; también fue identificado el serotipo de este nuevo aislado: serotipo 1 del virus de la lengua azul.

PALABRAS CLAVE: India – Inmunofluorescencia – Microscopía electrónica – Ovinos – Técnica de la inmunoperoxidasa – Virus de la lengua azul.

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