Isolation of bluetongue virus from Culicoides sp. in India

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Summary: Over 200 non-engorged midges (Culicoides) were caught in the vicinity of a flock of sheep infected with bluetongue virus (BTV) serotype 1. An isolate of virus, recovered from the midges by inoculating cultures of BHK-21 cells, was confirmed as BTV by using the double immunodiffusion test.

KEYWORDS: Bluetongue virus - Culicoides - India - Insect vectors - Orbivirus.

INTRODUCTION

Bluetongue (BT) is an insect-borne orbivirus infection of ruminants characterised by high fever, hyperaemia, oedema, ulcers in the oral cavity and reddening of the coronary bands. Sheep are affected more often than cattle, goats and other ruminants. The most important vector of BTV in Africa and the Near East is Culicoides imicola while in North America, C. variipennis prevails. Serological evidence for the presence of BT infection in India has been reported by various workers (1, 8, 9). Recently, Jain et al. (5) succeeded in isolating BTV from sheep blood. However, there seems to be no report of isolation of BTV from an insect vector in India. The present communication reports the isolation of BTV from Culicoides species for the first time in India.

MATERIALS AND METHODS

Insects

Insects were collected at an organised sheep breeding farm near Hisar (Haryana State) in July. The minimum and maximum temperatures were 26.1°C and 35.1°C respectively. The mean relative humidity and total rainfall during July were 85% and 132.6 mm, respectively. The species of Culicoides was not identified.

Virus isolation

Pools of midges (Culicoides sp.) numbering up to 200 were ground in a pestle and mortar with sterilised sea sand. After grinding, 10 ml sterilised Hank’s balanced salt solution (HBSS) at pH 7.2 was added. The sample was sonicated at 110 watts for 60 seconds. The suspension was then centrifuged at 3,000 rpm in a refrigerated...
centrifuge for 30 minutes. The supernatant was passed through a millipore filter (0.45 µm pore diameter). The filtered material was then inoculated in BHK-21 cells using the Mellor and Boorman method (7). Development of a cytopathic effect (CPE) was observed daily for up to eight days after inoculation. The isolate was titrated after the fifth passage in BHK-21 cells using the Reed and Muench method (10).

**Double immunodiffusion test**

The presence of BTV, suspected from a CPE in BHK-21 cells in the second passage, was tested by the double immunodiffusion test. The method described by Jochim and Chow (6) was followed. Standard reference BTV group-specific serum was placed in the central well. The second-passage isolate and standard BTV antigen were placed in adjacent peripheral wells. Reference antigen and antiserum were generously supplied by Dr. Michael M. Jochim (Denver, USA). One of the remaining two wells was filled with uninfected BHK-21 cell fluid (negative control) while the other was filled with culture medium. The slides were incubated in a humid chamber at 25°C for 72 hours. An isolate from the fifth passage was also tested by this method.

**RESULTS AND DISCUSSION**

Over 200 non-engorged female *Culicoides* were collected in the vicinity of sentinel sheep flocks. The triturated and sonicated filtrate of *Culicoides* produced a slight CPE in the second passage in BHK-21 cells, after incubation for up to eight days. In subsequent passages, there was extensive rounding of cells with increased refractivity of the cell monolayer, and detachment of the cell sheet from the glass surface after 48-72 hours of incubation at 37°C. The titre of the virus isolate was $10^{5.2}$ after the fifth passage in BHK-21 cells.

The isolate was tested against standard group-specific BTV reference serum at the second and fifth passages. A line of identity was seen between the isolate and the standard group-specific soluble antigen of BTV within 48 hours. This isolation from midges confirmed a previous isolation of BTV from sheep of the same flock by Jain *et al.* (5). Others have reported the presence of BTV in various species of *Culicoides* from many countries (2, 3, 4). However, there seems to be no earlier report of isolation of BTV from an insect vector in India. Thus, it is established that *Culicoides* may be the vector of BTV in India. Non-engorged female *Culicoides* were selected for isolation of the virus, because engorged midges may contain virus present in the blood of BTV-infected animals, which would not prove that the midges act as vectors. Confirmation of vector status of *Culicoides* would require artificial feeding of insects with a blood meal containing the virus and, after several days of feeding, testing the capability of infected midges to transmit BTV to the susceptible hosts.

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Résumé : Plus de 200 insectes Culicoides, non gorgés de sang, ont été capturés au voisinage d’un troupeau de moutons infecté par le sérotype 1 du virus de la fièvre catarrhale. L’inoculation de cultures de cellules BHK-21 a permis d’isoler une souche de virus à partir de ces insectes ; l’épreuve d’immunodiffusion double a confirmé qu’il s’agissait du virus de la fièvre catarrhale.

MOTS-CLÉS : Culicoides - Inde - Insectes vecteurs - Orbivirus - Virus de la fièvre catarrhale du mouton.


Resumen: Se capturaron más de 200 insectos Culicoides, que no habían absorbido sangre, a proximidad de un rebaño de carneros infectados por el serotipo 1 del virus de la lengua azul. Mediante la inoculación de cultivos celulares BHK-21, se pudo aislar una cepa de virus a partir de estos insectos y la prueba de inmunodifusión doble confirmó que se trataba del virus de la lengua azul.

PALABRAS CLAVE: Culicoides - India - Insectos vectores - Orbivirus - Virus de la lengua azul.

REFERENCES


