家畜衛試所研報 No.39：81～84(2003)

Identification of bluetongue virus in goats in Taiwan

LJ TING*, MS LEE, TS HUANG, CC HUANG, ST KUO, FLEE,

MH JONG, JR SHIAU, SYLIN

Bluetongue is an arthropod-borne, infectious viral disease of domestic and wild ruminants. The disease occurs throughout tropical and temperate regions where the vectors such as *Culicoides* species, responsible for transmitting the virus are present (Gibbs and Greiner 1994, Osburn 1994). Sheep are the most severelv affected livestock species, whereas cattle and goats are usually asymptomatic reservoir hosts (MacLachlan 1994). To date, at least 24 serotypes of bluetongue virus (BTV) have been identified. Several methods including virus neutralisation (Howell and others 1970), plaque neutralisation (Davies and Blackburn 1971), plaque inhibition (Afshar 1994) and fluorescence inhibition (Blackwell and Lunt 1996) have been developed for serotyping. Recently, genetic typing by reverse transcribed PCR (RT-PCR) and nucleotide sequencing was also used to differentiate various serotypes of BTV in Australia and the USA (McColl and Gould 1991, Wilson and chase 1993). This short communication deseribes the isolation and identification of BTV from goats in Taiwans, it is also the first repotr of BTV in clonically healthy goats in Taiwan.

In May 2003, the goat herds on Kinmen Island were surveyed serologically for bluetongue with a competitive ELISA kit purchased from UK. Serum samples were collected from four goat farms, which were found to be seropositive at reates of 33 per cent(three of nine). 81 per cent (13 of 16), 100 per cent (four of four) and 100 per cent (18 of 18), respectively. Subsequently, virus isolation was performed on a total of 22 heparinized whole blood samples from seropositive animals. The method of virus isolation was as described by Clavijo and others (2000). Briefly, erythrocytes were washed three times with Dulbecco's phosphate-buffered saline and the washed cells were frozen and thawed repeatedly. A volume of 0.1 ml of the lysate was imtravenously inoculated on 11-day-old embryonated chicken egg. The inoculated eggs were incubated at 33℃ and observed daily for up to seven days. Embryos that died between the third and fifth days after inoculation were collected and their viscera, including the brain, liver, spleen, heart and lungs, were harvested and homogenised with minimum essential medium alpha to produce a 10 per cent (w/v) suspension, the suspension was centrifugated and inoculated onto BHK-21 cells. A Cytopathic effect was observed during the third and fourth day after inoculation. The supernatant of infected BHK-21 cells was examined by electron mieroscopy, and revealed

*抽印本索取作者
行政院農業委員會家畜衛生試驗所

particles with a typical morphology of Reoviridae. The virus was submitted to the CSIRO Australian Animal Health Laboratory, Geelong, Victoria, Australia, for esrotyping, this confirmed that the virus isolated from Kinmen, named BTV/KM strain, belongs to BTV serotype 2.

Viral sequences of the BTV/KM strain, corresponding to nucleotides 11 to 284 of the RNA 6 genome (OIE 2000) and the full length sequence of the L2 gene, were amplified from the supernatant of infected cell culture (Ohashi 2002). The sequence data of the BTV-L2 gene was submitted to GenBank (accession number AY462225). Sequence analysis and phylogenetic studies of the L2 gene showed that the BTV/KM strain was clustered together with BTV serotype 2 strains from other areas, mainly Italy isolates (AJ430057-62), a Corsica isolate (AF256601) and an isolate from the USA(M21946); the V440 strain (AF135218) from China was genetically closest to Kinmen isolate with approximately 95 per cent nucleotide identity. This result supported the suggestion that identification of BTV serotypes by analysis of the sequence of genomic segment 2 is possible (Maan and others 2003).

It is not known for how long BTV has been presented in Taiwan. Kinmen Island is geographically closed to the Huijin Province of mainland China, and is separated from the mainland by only 4 km of sea. The arthropod vectors of BTV, such as *Culicoides* species, could easily be transported from the mainland to the island on board ships. In addition, illegal trade of the asymptomatic livestock could also introduce the virus, Further investigation is required to determine whether this serotype or other serotypes of BTV are present in Taiwan.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the support of the CSIRO Australian Animal Health Laboratory at Geelong, Victoria, Australia, for assisting in the serotyping.

Keywords

Bluetongue, Taiwan, Goat, Serotype 2, Phulogenetic

Reference

1. AFSHAR, A. (1994) Bluetongue: laboratory diagnosis. *Comparative Immunology, Microbiology and Infectious Diseases* 17, 221-242

2. BLACKSELL, S.D.&LUNT, R.A.(1996) Asimplified fluorescence inhibition tests for the serotype determination of Australian bluetongue viruses, *Australian Veterinary Journal* 73, 33-34

3. CLAVIJO, A., HECKERT, R.A., DULAC, G.C.& AFSHAR, A. (2000) Isolation and identification of bluetongue virus. *Journal of Virological Methods* 87, 13-23

4. DAVIES, F.G.& BLACKBURN, N.K.(1971) The typing of bluetongue virus. *Research in Veterinary Science* 12, 181-183

5. GIBBS, E.P.J.& GREINER, E.C. (1994) The epidemilolgy of bluetongue. *Comparative Immunology, Microbiology and Infectious Diseases* 17,207-220

6. HOWELL, P.G., KUMM, N.A.& BOTHA, M.J. (1970) The application of improved techniques to the identification of strains of bluetongue virus. *Onderstepoort Journal of Veterinary Research* 37,59-66

7. MAAN, S., MAAN, N., O'HARA, R., SAMUEL, A.R., MEYER, A., RAO, S.& MERTENS, P.P.C. (2003) Complete sequence analysis and compatison of genome segment 2 (encoding outer capsid protein VP2) from representative isolates of the 24 BTV serotypes. OIE Bluetongue International Symposium, Taormian, Italy, 22.

8. MACLACHLAN, N.J. (1994) The pathogenesis and immunology of bluetongue virus infection of ruminants. *Comparative Immunology, Microbiology and Infectious Diseases* 17, 197-206

9. MCCOLL, K.A.& GOULD, A.R. (1991) Detection and characterization of bluetongue virus using the polymerase chain reaction. *Virus Research* 21, 19-34

10. Office International des Epizooties (OIE)(2000) Manual of Standares for Diagnostic Tests and Vaccines, Chapter 2.1.9. Bluetongue.

11. OHASHI, S., YOSHIDA K., YANASE T.& TSUDA T. (2002) Analysis of intratypic variation evident in an Ibaraki virus strain and It's epizootic hemorrhagic disease virus serogroup. *Journal of Clinical Microbiology* 40,3684-3688

12. OSBURN, B.I. (1994) Bluetongue virus. *Veterinary clinics of North America: food animal practice* 10, 547-560

13. WILSON, W.C.& CHASE, C.C.L. (1993) Nested and multiplex polymerase chain reactions for the identification of bluetongue virus infection in the biting midge, *Culicoides variipennis. Journal of Virological Methods* 45, 39-47

台灣首例自山羊分離出藍舌病病毒

丁履紉*、李敏旭、黃天祥、黃金城、郭舒亭
李　璠、蕭終融、鍾明華、林士鈺

行政院農業委員會家畜衛生試驗所

摘要　藍舌病（Bluetongue）爲節肢動物媒介病毒性疾病，主要感染反芻獸。綿羊感染症狀比較嚴重，牛和山羊多爲不顯性感染。2003年藍舌病監測計畫，發現金門縣送檢羊隻血清藍舌病抗體陽性率偏高，分別爲33%(3/9)，81%(13/16)，100%(4/4)和100%(18/18)。因些針對這些羊場採集22個抗凝血樣品進行病毒分離。首先，將紅血球以 PBS 清洗3次，冷凍解凍後靜脈接種11-13日齡雞胚胎蛋。培養7日後，取胚胎之腦、心、肝、脾、腎以 MEM 培養液製成10倍臟器乳劑。將乳劑接種 BHK 細胞株，觀察 CPE 之有無。出現CPE的細胞上清液以電顯觀察，結果爲Orbivirus屬病毒。同時以BTVNS3引子，利用RT-PCR反應可以得到235bp的產物。病毒株（BTV-KM）送至澳洲參考實驗室，進行型別鑑定爲第2血清型。解讀完成之L2基因序列，以基因樹狀圖分析發現BTV-KM，明顯可以分類爲第2血清型，而且與大陸之V440株最接近，約有95%的核酸相似性。

---

*抽印本索取作者

本文轉載自 Vet Rec 2004: 155