

## Frequency of serological cross-reactions between Ibaraki and bluetongue viruses using the agar gel immunodiffusion test

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### Summary

The frequency of serological cross-reactions between Ibaraki (IB), and bluetongue (BT) viruses using the agar gel immunodiffusion (AGID) test was investigated. The percentage of IB neutralisation-positive bovine serum samples that were positive to the BT AGID test was 42.5%; 12.2% of the BT AGID-positive serum samples and 2.5% of the BT AGID-negative serum samples were positive to the IB AGID test. When the BT competitive ELISA (c-ELISA) was used, these cross-reactions disappeared. These results indicate that serum samples from areas in which IB is epidemic are often positive against the BT AGID test, but negative against the BT virus neutralisation test (VNT). To obtain specific BT surveillance results in these IB endemic areas, the AGID-positive results should be confirmed using the c-ELISA or VNT.

### Keywords

Agar gel immunodiffusion test – Bluetongue virus – Cattle – Competitive enzyme-linked immunosorbent assay – Cross-reaction – Diagnosis – Epizootic haemorrhagic disease – Ibaraki virus – Japan – Sheep – Virus neutralisation test.

### Introduction

Ibaraki (IB), a member of the epizootic haemorrhagic disease (EHD) serogroup, is endemic in Japan (5, 7). The agar gel immunodiffusion test (AGID) (13) is performed to survey for BT antibody in Japan. BT AGID-positive results are sometimes observed in areas of Japan in which IB is endemic and this makes interpretation of BT surveillance results difficult. Cross-reactions between bluetongue (BT) and EHD virus serogroups have been reported (9, 10, 11, 12, 14, 15, 16, 17). These cross-reactions were apparent in the serum samples from experimentally immunised animals (9, 10, 11, 14, 16). However, the frequency of cross-reactions of BT virus (BTV) or IB virus (IBV)-positive field samples against the IB and BT AGID tests is not clear (11, 17). The authors attempted to clarify the significance of BT and IB AGID test cross-reactions. Furthermore, we evaluated the cross-reaction between IB and BT in the competitive enzyme-linked immunosorbent assay (c-ELISA) (8, 14).

### Materials and methods

#### Serum samples

Forty serum samples from serologically positive IB cattle were collected in 1997 from the Hyogo Prefecture in Japan to evaluate the serological cross-reactions with BT. In 1994, 202 serum samples were collected from serologically positive BT herds in the Tochigi Prefecture to check serological cross-reactions with IBV. IB was not epidemic in the Tochigi Prefecture. Of the 202 samples, 125 were from cattle and 77 from sheep. Of the cattle sera, 65 were positive for BTV neutralising antibody and 60 negative; of the sheep sera, 58 were positive for BTV neutralising antibody and 19 negative.

#### Serological tests

Sera from animals that were suspected of being infected with IBV were tested to determine the IBV and BTV neutralising antibody titres and the reactions to the BT and IB AGID tests. In addition, the percentage inhibition of the samples to the BT

c-ELISA was determined. Serum from animals that were suspected of being infected with BTV were tested to determine the BTV neutralising antibody titres, the reactions to the BT and IB AGID tests and the percentage inhibition of samples to the BT c-ELISA. The serological test procedures were as described in the OIE *Manual of standards for diagnostic tests and vaccines* (13).

**Virus**

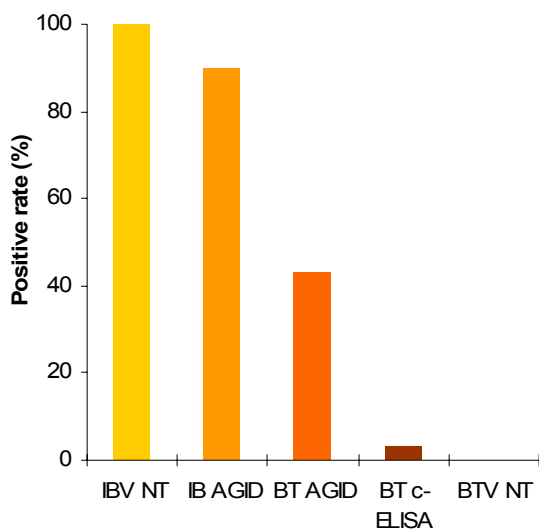
BT virus serotype 21 or IB virus (EHDV type 2) were used in the virus neutralisation test (VNT).

**Results**

**Cross-reaction of Ibaraki-positive serum using the bluetongue agar gel immunodiffusion test**

Serum samples that were positive for IBV neutralising antibody had the following reactions:

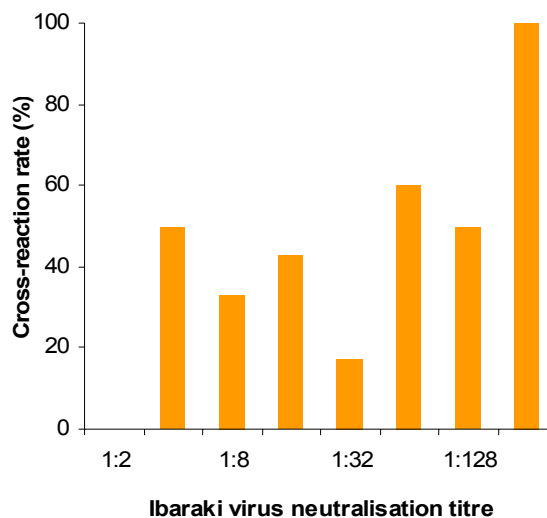
- a) IB AGID test: 90% (36/40)
- b) BT AGID test: 42.5% (17/40)
- c) BT c-ELISA: 2.5% (1/40)
- d) BT VNT: 0% (0/40) (Fig. 1).



**Figure 1**  
Rates of cross-reaction of Ibaraki-positive serum using serodiagnosis for bluetongue virus  
Of the Ibaraki virus neutralisation-positive and BTV neutralisation-negative serum samples, 42.5% were bluetongue agar gel immunodiffusion-positive  
Only one of the Ibaraki virus-neutralisation positive sera (2.5%) was positive using the BT c-ELISA

Thus, 42.5% of Ibaraki-positive serum samples, which were negative to the BT VNT, were positive to the BT AGID; only one of the IB-positive serum samples was positive to the BT c-ELISA.

As shown in Figure 2, only a few samples with low IBV neutralising antibody titres had cross-reactions on the BT AGID test; however, all of the samples that had NT titres of more than a 1:256 were BT AGID positive.



**Figure 2**  
Cross-reaction of Ibaraki virus infected serum classified by Ibaraki virus neutralisation on bluetongue virus agar gel immunodiffusion  
Percentage of serum samples with Ibaraki virus neutralising antibody titres of greater than 1:4 that were positive to the bluetongue agar gel immunodiffusion test

**Cross-reaction of bluetongue virus neutralisation-positive serum using the Ibaraki immunodiffusion test**

Of the 125 bovine serum samples, 60 were negative (<1:2) to the BT VNT and 65 were positive (>=1:2). One BT VNT-negative bovine serum sample and five BT VNT-positive bovine serum samples were positive to the IB AGID. The rates of cross-reactions were: 4.8% (6/125) in total; 7.7% (5/65) of the bovine BT VNT-positive samples were IB AGID-negative and 1.7% (1/60) BT VNT-negative serum samples were positive to the IB AGID (Table I).

**Table I**  
Cross-reaction of bovine sera from bluetongue-infected herds using the Ibaraki agar gel immunodiffusion test  
Numbers indicate real number of bovine serum samples

BTV-negative serum 60		BTV-NT positive serum 65	
Ibaraki AGID		Ibaraki AGID	
Positive	Negative	Positive	Negative
1	59	5	60
(1.7%)	(98.3)	(7.7%)	(92.3%)

BTV bluetongue virus  
NT neutralisation  
AGID agar gel immunodiffusion

Of the 77 sheep serum samples, 19 were BT VNT-negative (<1:2) and 58 were positive (>1:2). One BT VNT-negative ovine serum sample and ten BT VNT-positive ovine serum samples were positive to the IB AGID. The rates of cross-reaction of the ovine samples between BT VNT and IB AGID were 14.3% (11/77) in total; 5.3% (1/19) of the BT VNT-negative samples were positive and 17.2% (10/58) of ovine BT VNT-positive serum samples were positive to IB AGID (Table II).

**Table II**  
Cross-reaction of ovine sera from bluetongue-infected herds using the Ibaraki agar gel immunodiffusion test  
Numbers indicate real numbers of ovine serum samples

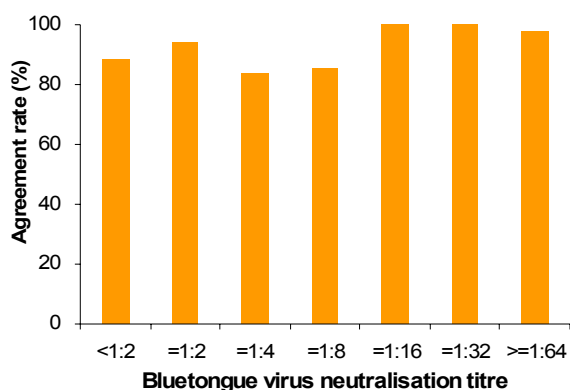
BTV-negative serum 19		BTV-NT positive serum 58	
Ibaraki AGID		Ibaraki AGID	
Positive	Negative	Positive	Negative
1	18	10	48
(5.3%)	(94.7%)	(17.2%)	(82.7%)

BTV bluetongue virus

NT neutralisation

AGID agar gel immunodiffusion

The agreement between BT VNT and both the BT c-ELISA and BT AGID test was 88.1%, and the agreement between BT c-ELISA and BT AGID was 98.0% (Fig. 3).



**Figure 3**  
Agreement rate between bluetongue c-ELISA and bluetongue agar gel immunodiffusion classified by bluetongue virus neutralisation titre

## Discussion

Cross-reactions between the BTV and EHDV serological tests have been documented for serum samples from immunised animals. A high incidence of BT AGID-positive serum samples from North American wild ruminants has been reported (12, 17); these positive results may be due to EHDV infection

(9). However, the cross-reactions of field samples to the IB and BT AGID tests is unclear (11). Our results indicated that serum from IB VNT-positive animals were frequently positive to the BT AGID. Furthermore, serum samples with low IBV neutralising antibody titres showed cross-reactions to the BT AGID test. IB-positive serum samples were rarely positive to the BT c-ELISA. These results indicate that a more specific test, such as c-ELISA, should be used for BT surveys in EHDV-endemic areas. As the serum samples with high neutralising antibody titres (more than 1:256) cross reacted with the BT AGID, more cross-reactions can be expected following an epidemic of IB when the antibody titres would be expected to be higher.

BT-positive serum samples quite frequently were positive to the IB AGID but there were fewer IB-positive samples using the BT AGID test. These results indicate that cross-reactions between IB and BT on AGID do occur. As previously reported (1, 2, 3, 4, 6, 14), results of the BT c-ELISA were very similar to the results of the BT VNT and the results of the BT c-ELISA were more precise than the BT AGID (1, 2, 3, 4, 6). Although the agreement between c-ELISA and BT AGID was 98%, BT c-ELISA is still recommended for serological surveys to detect BTV infection in EHDV-endemic areas.

## Conclusion

Specific serodiagnostic techniques, such as c-ELISA or BT VNT, should be used for BT surveillance in IBV-epidemic areas because IB-positive serum samples may result in false-positive BT AGID test reactions.

## References

- Anderson J. (1984). – Use of monoclonal antibody in a blocking ELISA to detect group specific antibodies to bluetongue virus. *J. Immunol. Methods*, **74**, 139-149.
- Afshar A., Thomas F.C., Wright P.F., Shapiro J.L., Shettigara P.T. & Anderson J. (1987). – Comparison of competitive and indirect enzyme-linked immunosorbent assays for detection of bluetongue virus antibodies in serum and whole blood. *J. Clin. Microbiol.*, **25**, 1705-1710.
- Afshar A., Thomas F.C., Wright P.F., Shapiro J.L. & Anderson J. (1989). – Comparison of competitive ELISA, indirect ELISA and standard AGID tests for detecting bluetongue virus antibodies in cattle and sheep. *Vet. Rec.*, **124**, 136-141.
- Afshar A., Eaton B.T., Wright P.F., Pearson J.E., Anderson J., Jeggo M. & Trotter H.C. (1992). – Competitive ELISA for serodiagnosis of bluetongue: evaluation of group-specific monoclonal antibodies

- and expressed VP7 antigen. *J. Vet. Diag. Invest.*, **4**, 231-237.
5. Gorman B.M., Taylor J. & Walker P.J. (1983). – Orbiviruses. In *The Reoviridae* (W.A. Joklik, ed.). Plenum Press, New York, 287-357.
  6. House C., House J.A. & Berninger M.L. (1990). – Detection of bluetongue group-specific antibody by competitive ELISA. *J. Vet. Diag. Invest.*, **2**, 137-139.
  7. Inaba Y. (1975). – Ibaraki disease and its relation to bluetongue. *Aust. Vet. J.*, **51**, 178-185
  8. Lunt R.A., White J.R. & Blacksell S.D. (1988). – Evaluation of a monoclonal antibody blocking ELISA for the detection of group-specific antibodies to bluetongue virus in experimental and field sera. *J. Gen. Virol.*, **69**, 2729-2740.
  9. Moore D.L. (1974). – Bluetongue and related viruses in Ibadan, Nigeria: serologic comparison of bluetongue, epizootic hemorrhagic disease of deer, and Abadina (Palyam) viral isolates. *Am. J. Vet. Res.*, **35**, 1109-1113.
  10. Moore D.L. & Lee V.H. (1972). – Antigenic relationship between the virus of epizootic haemorrhagic disease of deer and bluetongue virus. *Archiv. Gesamt. Virusforsch.*, **37**, 282-284,
  11. Moore D.L. & Kemp G.E. (1974). – Bluetongue and related viruses in Ibadan, Nigeria: serologic studies of domesticated and wild animals. *Am. J. Vet. Res.*, **35**, 1115-1120.
  12. Murray J.O. & Trainer D.O. (1970). – Bluetongue virus in North American elk. *J. Wildl. Dis.*, **6**, 144-148.
  13. Office International des Épizooties (OIE) (2002). – Bluetongue, Chapter 2.1.9. In *Manual of standards for diagnostic tests and vaccines*. OIE, Paris ([oie.int/eng/normes/mmanual/A\\_00032.htm](http://oie.int/eng/normes/mmanual/A_00032.htm) accessed on 13 August 2004).
  14. Reddington J.J., Reddington G.M. & MacLachlan N.J. (1991). – A competitive ELISA for detection of antibodies to the group antigen of bluetongue virus. *J. Vet. Diag. Invest.*, **3**, 144-147.
  15. St George T.D., Cybinski D.H., Standfast H.A. & Gard G.P. (1983). – The isolation of five different viruses of the epizootic haemorrhagic disease of deer serogroup. *Aust. Vet. J.*, **60**, 216-217.
  16. Thomas F.C. & Miller J. (1971). – A comparison of bluetongue virus and EHD virus: electronmicroscopy and serology. *Can. J. Comp. Med.*, **35**, 22-27.
  17. Trainer D.O. & Jochim M.M. (1969). – Serologic evidence of bluetongue in wild ruminants of North America. *Am. J. Vet. Res.*, **30** (11), 2007-2011.