

# Epidemiological observations on bluetongue in sheep and cattle in Japan

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## Summary

Bluetongue (BT) first occurred in Japan between late August and October 1994 in 23 cattle in three prefectures of the northern Kanto region, and between the end of October and mid-November in 23 Suffolk sheep in the same region. The affected cattle had fever, deglutitive difficulty, hypersalivation, facial oedema, scabbing of the corner of the mouth and dysphagia. The BT virus (BTV) was isolated from blood cells of the affected sheep. Surveillance for BTV antibody conducted by prefectures in the affected region has detected seroconversion to BTV in some prefectures every year thereafter. In the autumn of 2001, again in the northern Kanto region, 45 sheep developed BT, and BTV was isolated. It is considered important that Japan has imported numerous cattle from Australia, the United States of America (USA), and New Zealand every year. In particular, BTV was isolated from cattle imported from the USA during quarantine although some of the serotypes isolated are not present in the USA. Furthermore, BTV is not present in New Zealand. The third RNA segment encoding the serogroup-specific VP3 protein of Japanese BTV isolates and reverse transcriptase-polymerase chain reaction (RT-PCR) positive blood cells was amplified by RT-PCR. Molecular phylogenetic analysis of the third RNA segment based on the sequence homology of the PCR products led to the classification of Japanese BTV isolates into two major groups.

## Keywords

Bluetongue – Cattle – Epidemiology – Japan – Molecular epidemiology – Sequencing – Sheep.

## Introduction

Seroepidemiological surveillance conducted in 1974 by Miura *et al.* (4) showed that domestic cattle in the Kyushu and Okinawa regions of Japan had antibodies to BTV serotypes 1, 12 and 20. In July and August 1979, cattle seroconverting to these serotypes were also confirmed on Miyakojima Island in the Okinawa Prefecture. Since the later national arbovirus surveillance conducted in Japan detected seroconversion in sentinel domestic cattle in all prefectures in the Kyushu and Okinawa Prefectures, subclinical infection with BTV appears to be prevalent in these regions in the absence of obvious bluetongue (BT) disease.

## Materials and methods

### Collection of blood from cattle and sheep in epidemic areas

Heparinised blood and serum for antibody detection were collected from 170 sheep including those that developed BT. Blood was centrifuged at 3 000 rpm at 4°C for 10 min, and separated into plasma and blood cells to isolate viruses. Blood cells were washed with 4°C phosphate-buffered saline (PBS) three times. Viruses were isolated using HmLu-1 cells derived from the baby hamster lung (5). In addition, blood cell suspensions were inoculated into the veins of 11-day-old embryonated chicken eggs, and chicken embryos and embryonic organs of eggs that died were inoculated onto Vero cells for virus isolation.

## Pathology

Affected cattle and sheep that died were autopsied, and organs were examined histologically using routine techniques.

## Seroepidemiological survey

Antibody surveys in areas of BT outbreaks, and a nationwide antibody survey in sentinel cattle, were performed by the neutralisation test in microplates as previously reported (4). The agar gel immunodiffusion (AGID) test for group-reactive antibodies was performed in accordance with the OIE *Manual*.

## Polymerase chain reaction and phylogenetic analyses

Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed as described by McColl *et al.* (2, 3). The third RNA segment (RNA3) encoding the serogroup-specific VP3 protein of Japanese BTV isolates and RT-PCR-positive blood cells was amplified by RT-PCR. Based on the sequence homology of the PCR products, a molecular phylogenetic tree of RNA 3 was constructed by the unweighted pair-group method using arithmetic means (7).

## Results

### Increase in imported cattle and isolation of BTV

In recent years, Japan has imported numerous cattle from the USA and Australia, and the number of cattle imported is on the increase (Table I). Thus, BTV has been isolated frequently from the blood of imported cattle during quarantine. In particular, from 121 Holstein cattle imported from the USA in September 1990, 33 strains of BTV were isolated; from 89 cattle imported in September 1995, 13 strains of BTV and 18 strains of epizootic haemorrhagic disease virus (EHDV); and from 123 cattle imported in November 1995, five strains of BTV and three strains of EHDV. The BTV isolates were of serotypes 4, 11, 13 and 20.

Table I  
Inspected cattle and articles, by exporting country

Exporting country	1998	1999	2000	2001	2002
United States of America	211	211	253	220	129
Australia	17 194	17 194	14 230	17 376	13 964
Canada	125	125	115	134	99
New Zealand	785	785	322	1 134	355
Other	8	8	0	0	0

Importantly, however, BTV serotypes 4 and 20 do not occur in the USA. The EHDV isolates were of serotypes 1 and 2. In contrast, no BTV has been directly isolated from the cattle imported from Australia. However, 7 strains of BTV were isolated from *Culicoides brevitarsis* collected in the quarantine house for imported cattle at the Okinawa Branch of the Animal Quarantine Station.

### Isolation of BTV from domestic cattle and sheep in Japan

Although BTV has been isolated from the blood of domestic cattle and *C. brevitarsis* since 1985, no outbreak of BT has been confirmed.

### Symptoms and histopathological findings in BT outbreaks in Japan and isolation of BTV

#### Cattle

Between August and October in 1994, a total of 23 Japanese Black beef cattle in three prefectures of the northern Kanto region developed BT with symptoms of fever, loss of appetite, hyper-salivation, facial oedema, scabbing of the corner of the mouth, and dysphagia. Histopathological examination of autopsy material from diseased cattle showed that the lesions were localised in the oesophagus, and consisted of hyaline degeneration and rupture of oesophageal striated muscle fibres, lymphocytic infiltration and regeneration of muscle fibres. No BTV was isolated from the blood of 199 cattle including the diseased cattle and other cattle on the same farm.

#### Sheep

A total of 23 sheep on a Suffolk sheep farm in the northern Kanto region developed BT between the end of October and mid-November 1994. Symptoms included fever, dysphagia, tongue ulceration and laminitis, which were similar to but slightly more severe than those in cattle. BTV was isolated from three of the diseased sheep and identified as serotype 21. Some 45 sheep on the same farm where sheep had developed BT in 1994 developed BT between late September and late November 2001. Breeding ewes and lambs were most affected and developed symptoms of fever, dysphagia, auricular oedema and laminitis. Five sheep died. Although the symptoms of the diseased sheep were similar to those in 1994, ulceration of the tongue and scabbing of the nasal arch were less severe. Serotype 21 was isolated from the blood of three diseased sheep.

**Seroepidemiology of BTV infection in Japan: yearly seroconversion rates in sentinel cattle in Japan**

Seroconversions in sentinel cattle throughout Japan between 1999 and 2002 are shown in Fig. 1. In western Japan in 1999, the seroconversion rate was approximately 30% in the Shimane and Hiroshima Prefectures, 10%-13% in the Okayama and Yamaguchi Prefectures, and as high as 71% and 36.4% in the Kumamoto (in Kyushu) and Okinawa Prefectures, respectively. The seroconversion rates in the Tochigi Prefecture (where an outbreak of BT in sheep was confirmed in 2001) and in the neighbouring Ibaraki and Fukushima Prefectures were 10.8% and 5%-6%, respectively. In 2001, BTV seroconversions occurred in numerous cattle in

western Japan: the conversion rates in Okayama, Ehime and Kochi were 44.8%, 43.8%, and 36%, respectively, confirming an epidemic to have occurred in some regions. In contrast, approximately 30% of cattle have seroconverted every year between 1999 and 2002 in the Okinawa Prefecture.

**Japanese BTV isolates: PCR-based detection and molecular phylogeny**

RT-PCR-positive cattle were detected in Fukushima in 1994, in Saga and Miyazaki in 1995, in Kagoshima and Okinawa in 1996, and in Tottori in 1997 during annual arboviral surveillance. Based on nucleotide sequence homology, the PCR products from Japanese BTV isolates and RT-PCR positive blood cells of cattle were broadly divisible into the 1994

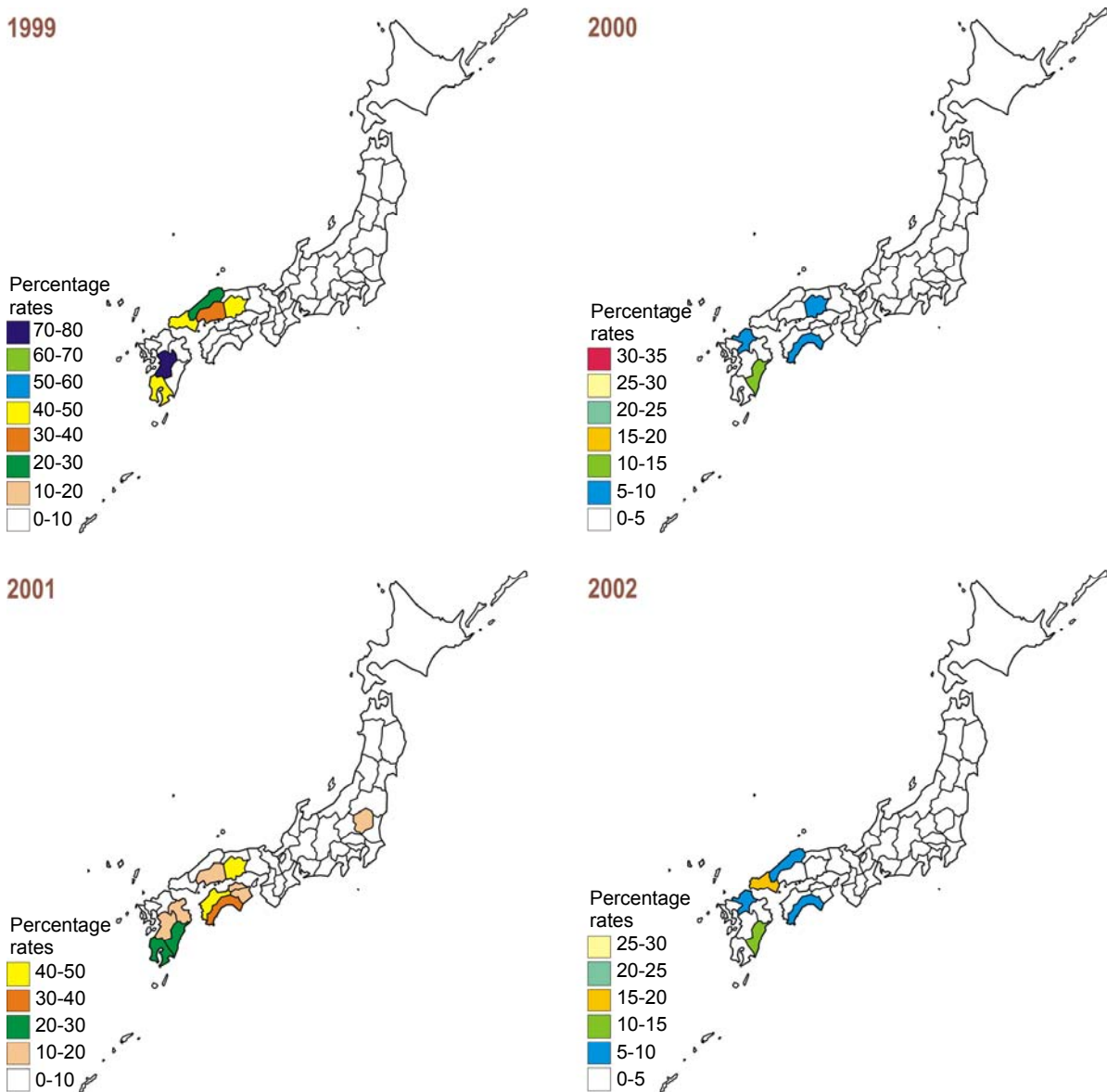


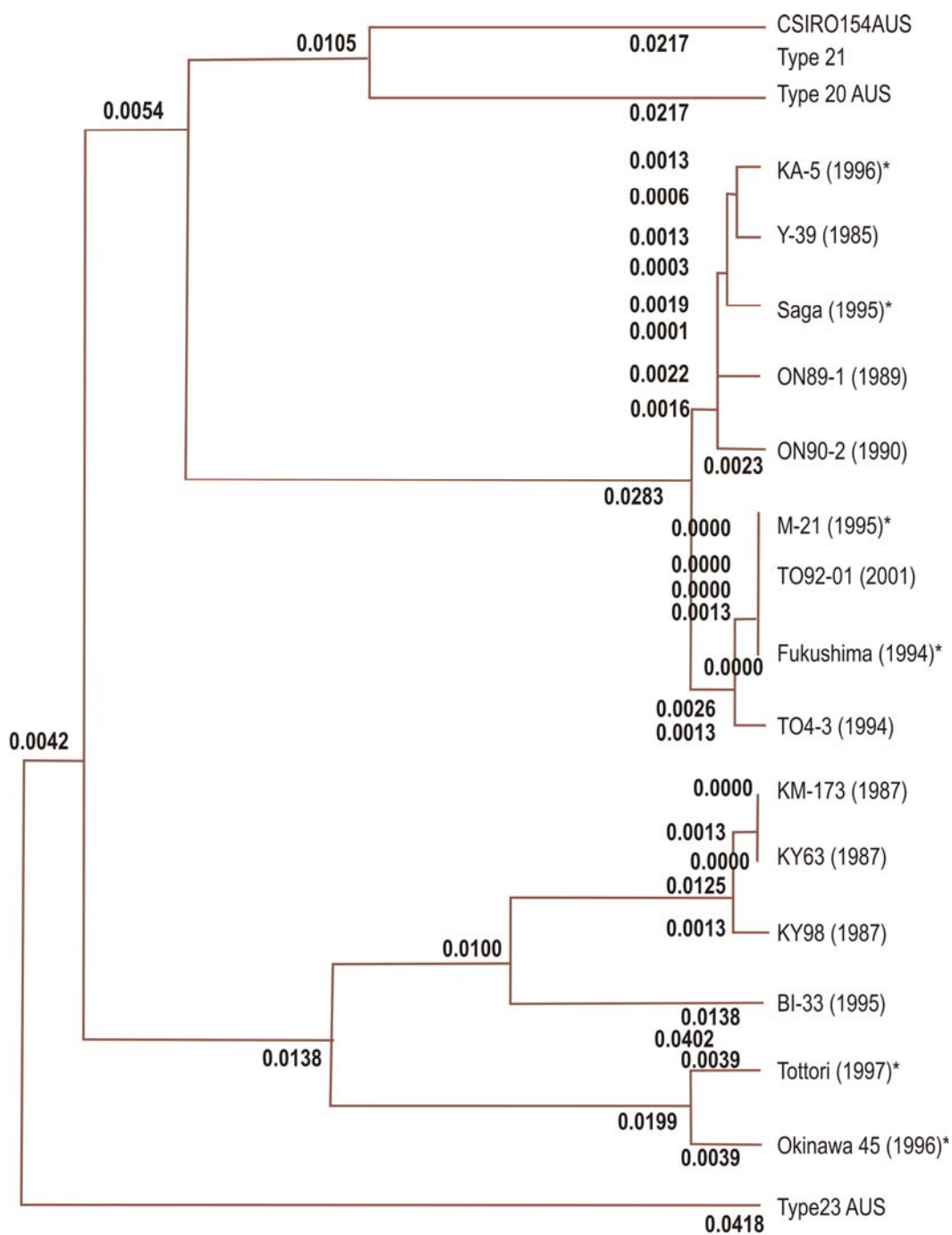
Figure 1  
Seroconversion percentage rates of sentinel cattle in Japan, 1999-2002

epidemic group and another group (Fig. 2). Many BTVs isolated to date were identified as belonging to serotype 21.

## Discussion

Although the epidemiological features of BT in Japan have not been well characterised, no cases of BT have occurred in Okinawa or other western

Japan prefectures where BTV has been isolated (8). However, the results of seroepidemiological surveillance suggest that subclinical or mild BTV infection of ruminants in Japan is prevalent almost every year, and that there are repeated and recurrent infections among domestic cattle and sheep in the affected regions. The serotype most frequently isolated to date is BTV-21, but its pathogenicity is not clear.



\* RNA from blood

Figure 2  
Phylogenetic tree based on sequence analysis of the amplified cDNA fragment of segment 3 of the bluetongue virus strains

In Japan, a BT outbreak first occurred in 1994 in the northern Kanto region (1) that had been regarded as a non-epidemic region. It is not clear why BT suddenly became prevalent in the mountainous area of the northern Kanto Region. Since BT developed in aged Japanese Black cattle, decreased immunological function and genetic factors cannot be excluded. The development of BT in sheep in the same region has attracted little attention because of the very small number of domestic sheep in Japan. The development of BT in cattle in a region, followed by development in domestic sheep in the same region, suggests that the virus spread from infected cattle to adjacent sheep. The second outbreak of BT among sheep on the same farm in 2001 might suggest that there had been persistent infection among sheep since the original outbreak in 1994, although an endemic cycle of infection or repeated incursions of BTV are also potential explanations. Although BTV has been isolated from imported cattle, the serotypes isolated have not always been endemic in the countries of export, suggesting infection after arrival in Japan; however, negotiations are needed with exporting countries over hygienic conditions including the quarantine system for imported cattle and sheep.

The Ibaraki virus, which is a member of the EHDV group of the *Orbivirus* genus, incurs into western Japan every 5 to 10 years, and infected cattle develop a 'BT-like disease' (6) with dysphagia due to paralysis of the oesophagus and pharynx; therefore, the differentiation of BT and similar diseases will become increasingly necessary in the future. Moreover, antigenically distinct strains of Ibaraki virus have caused many abortions in cattle, and their relationship with cattle dystocia has been suspected (9).

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