

Clinical signs of bluetongue virus serotype 8 infection in sheep and goats

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THE confirmation of bluetongue virus serotype 8 (BTV-8) in the Netherlands on August 17, 2006 marked the onset of the first outbreak of bluetongue in north-west Europe. BTV (*Orbivirus*, Reoviridae) is a vectorborne, double-stranded RNA virus with 24 known serotypes, which causes a non-contagious infectious disease in ruminants. The incursion of BTV-8 into Europe was preceded by epizootics of BTV serotypes 1, 2, 4, 9 and 16 in southern Europe since 1998 (Mellor and Wittmann 2002). For all these serotypes, clinical signs occurred in sheep and some species of wild ruminants, but no apparent signs were seen in goats and cattle. This is consistent with other outbreaks throughout the world (Mellor and Wittmann 2002, MacLachlan 2004). An animal affected with bluetongue may show congestion, oedema and haemorrhages caused by virus-mediated vascular injury. Hence, any combination of fever, serous to bloody nasal discharge, erosions and ulcers of the oral and nasal mucosa, oedema, coronitis and lameness, and weakness secondary to muscle necrosis may occur (MacLachlan 2004).

Although the presence of clinical signs in sheep and their absence in goats that were observed throughout the BTV-8 outbreak in north-west Europe in 2006 were in line with BTV outbreaks involving other serotypes, the presence of mild to severe signs of bluetongue in cattle were not. To the authors' knowledge, there are no reports of experimental BTV-8 infection in goats and only one recent study in sheep (Darpel and others 2007). The aim of this study was to assess the clinical signs of BTV-8 in sheep and goats and to describe basic epidemiological parameters.

To obtain an inoculum, a Texel sheep infected with BTV-8 in the field was bled a week after oral mucosa lesions and oedema of the head were first observed. Immediately after collection, the total cell fraction of the blood was washed five times to reduce BTV-specific antibodies and then suspended in phosphate-buffered saline (PBS). Five female Texel sheep and four female white Dutch dairy goats, all older than six months, were commercially sourced from different Dutch farms. Before BTV-8 infection, the sheep and goats were determined to be free from BTV RNA and BTV-specific antibodies, as

well as from Border disease virus RNA and antibodies. On the same day as collection, 24 ml of the inoculum was administered intravenously to three of the sheep and two of the goats; the remaining two sheep and two goats served as negative controls. The sheep were housed separately from the goats, both groups in high containment conditions.

Following inoculation, EDTA-blood and serum samples were collected from all the sheep and goats, daily during the first week after inoculation, every other day in the second week and once a week thereafter. These samples were tested using a real-time reverse transcriptase-PCR (A. Backx, R. G. Heutink, E. M. A. van Rooij, P. A. van Rijn, unpublished observations) for the detection of BTV RNA in EDTA-blood and a blocking ELISA (ID-VET) for the detection of BTV-specific antibodies in serum. Body temperature was recorded daily, with fever defined as above 40°C for sheep and above 40.5°C for goats. The animals were observed and examined physically every day, and any clinical signs were recorded. The administration of analgesic and antimicrobial treatment was allowed. If the clinical signs in the animals became so severe that their welfare was unacceptably compromised, they were euthanased. At the end of the experiment all the sheep and goats were euthanased by intravenous injection of a combination of 200 mg/ml embutramide, 50 mg/ml mebezoniumjodide and 5 mg/ml tetracaine hydrochloride (T61; Intervet).

Four days after infection, all the inoculated sheep and goats tested PCR positive for BTV. The inoculated sheep tested BTV antibody-positive at eight days postinfection and the inoculated goats at 13 days postinfection. All the inoculated animals apart from one goat developed fever. One inoculated sheep had a fever for four days, starting at four days postinfection, the others had fever for only one day, eight or nine days postinfection (Fig 1). Two inoculated sheep displayed severe signs of apathy, seromucous to bloody nasal discharge (Fig 2), dysphagia, dyspnoea, oedema of the head (Figs 2, 3), erosions and haemorrhages of the oronasal mucosa. These findings in sheep are in line with those described in a study by Darpel and others (2007). The inoculated goat with fever showed slightly milder signs than the sheep, consisting of generalised illness (Fig 4), apathy, dysphagia, diarrhoea and lameness; the other inoculated goat did not show any signs. All the controls tested BTV-negative by PCR and ELISA and did not demonstrate any clinical signs or fever.

The clinical signs of bluetongue that were observed after experimental infection of sheep with BTV-8 were consistent with the clinical signs that were reported most frequently from the field in 2006. The most prominent of these were fever, apathy, erosions of the oral mucosa, excessive salivation, dysphagia, oedema of the head and jaw, oedema of the lips and lameness (Elbers and others 2007). In contrast to field reports, clinical signs of bluetongue could be experi-

Veterinary Record (2007)
161, 591-593

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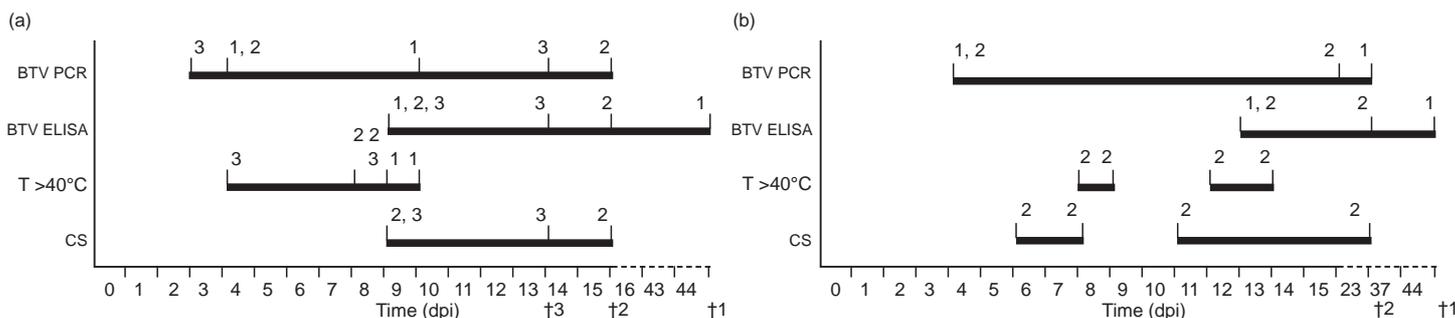


FIG 1: Onset and duration of positive test results for bluetongue virus (BTV) PCR, BTV ELISA, fever and recorded clinical signs of bluetongue in days postinoculation (dpi) with BTV serotype 8 for (a) sheep and (b) goats. The scale is discontinuous after 15 dpi. The numbers above the bars mark the duration for the individual animals. † indicates the death of the numbered animal. T Temperature, CS Clinical signs

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FIG 2: Sheep inoculated with bluetongue virus serotype 8, at 13 days postinfection, showing oedema of the face, muzzle and ears, conjunctivitis and bloody mucous nasal discharge



FIG 4: Bluetongue virus serotype 8 inoculated goat (in the background) displaying signs of general illness and depression



FIG 3: Control animals and bluetongue virus serotype 8 inoculated sheep were housed together; the sheep in the middle is showing signs of depression, oedema of the face and ears at 13 days postinfection

mentally produced in a goat: fever, signs of general illness, apathy, dysphagia, diarrhoea and lameness were observed. A possible reason for the difference might be that the route of infection used in this study, intravenous injection, was different from the natural route of infection by the bite of an infected *Culicoides* species midge. Hence, goats in the field might not have become infected with BTV-8 because of vector preferences or the type of husbandry systems. Alternatively, goats in the field may have been infected with BTV but not tested for the virus because they showed no clinical signs, or

because signs that were seen were not those typically related to bluetongue. Field research is required to assess the prevalence of bluetongue in goats.

ACKNOWLEDGEMENTS

The authors thank the animal caretakers who participated in the study, for their assistance.

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Veterinary Record 2007 161: 591-592

doi: 10.1136/vr.161.17.591

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