Clinical syndromes associated with the circulation of multiple serotypes of bluetongue virus in dairy cattle in Israel


From 2008 to 2011, seven distinct bluetongue virus (BTV) serotypes (BTV-2, BTV-4, BTV-5, BTV-8, BTV-15, BTV-16 and BTV-24) have been identified to be circulating in diseased sheep and cattle in Israel. This paper describes the array of clinical manifestations caused by BTV in cattle in Israel. Each set of clinical manifestations has been categorised as a syndrome and six distinct clinical syndromes have been observed in dairy cattle: ‘footrot-like syndrome’, ‘sore nose syndrome’, ‘subcutaneous emphysema syndrome’, ‘red/rough udder syndrome’, ‘bluetongue/epizootic haemorrhagic disease systemic syndrome’ and ‘maladjustment syndrome’.

BLUETONGUE virus (BTV) is the type species of the genus Orbivirus, within the family Reoviridae. Currently, 24 different serotypes of BTV have been identified according to the specificity of interactions between neutralising antibodies and the more variable components of the virus’s outer capsid, particularly the VP-2 protein (Roy and others 1992). BTVs are transmitted by the adults of certain Culicoides species and can infect all ruminants (Schwartz-Cornil and others 2008, Maclachlan and others 2009).

Clinical signs of BTV infection are usually confined to sheep, especially improved meat and wool breeds, which may develop a severe haemorrhagic disease called bluetongue (BT). Elbers and others (2008a, b, c, 2009) introduced the concept of BT-attributed clinical signs during their study of the epidemiology of BTV-8 infection across Europe. These signs included fever, anorexia, dysphagia, ulcerative and necrotic lesions of the oral mucosa, hyperaemia and oedema of the conjunctival mucosa, sore muzzle, hyperaemia of the teats and udder, haemorrhage, dehydration and lameness (Jeggo and others 1987, Veronesi and others 2005, Darpel and others 2007, Eschbaumer and others 2010, Pardon and others 2010). From August 2008 to October 2010, five BTV serotypes (BTV-4, BTV-5, BTV-8, BTV-16 and BTV-24) were shown to be circulating in Israel (Brenner 2009, Brenner and others 2010); all the isolates were obtained from diseased sheep and cattle. During this period, clinical manifestations caused by BTV were observed for the first time in Israeli cattle populations (Brenner 2009). Two additional serotypes, BTV-2 (previously identified in Israel in 2004) and BTV-15 (previously identified in Israel in 2006), were discovered in samples taken during outbreaks of BT in late 2010 and early 2011.

The aim of the present paper is to describe the array of clinical manifestations observed in cattle during the 2008 to 2010 BTV outbreaks in Israel (Brenner and others 2010). The clinical manifestations described are broadly similar to those already attributed to BTV infection in cattle in Europe, especially during the BTV-8 outbreaks (Elbers and others 2008a, b, c, 2009); however, those authors described clinical signs associated with a single BTV strain (BTV-8), whereas the clinical signs described in the present study represent a ‘cluster’ of signs caused by multiple co-circulating serotypes of BTV.

Materials and methods
Animals and farms
Clinical investigations were carried out on 11 large dairy farms, each holding approximately 250 lactating cows. The location of these farms is shown in Fig 1. During farm visits, the morbidity and mortality were recorded and the case histories were obtained from the farmers and the local veterinary practitioners.

Sample collection and laboratory testing for BTV
Blood samples (EDTA and whole blood) and internal organs from animals showing clinical signs of BT were initially tested at the Kimron Veterinary Institute (KVI), Israel, and the samples that tested positive were subsequently sent to the Institute for Animal Health, Pirbright, UK, where they were tested by group-specific PCR (Shaw and others 2007) to confirm the presence of BTV, and then serotype-specific PCR (Laboratoire Service International) to identify the BTV serotype. Attempts were also made to isolate virus from the samples (Oura and others 2009). Serum samples were tested by competitive ELISA (CELISA; ID Vet), and in some cases a serum neutralisation test was carried out in order to identify the serotype (Haig and others 1956). Only cases in which BTV was diagnosed in the laboratory, by either PCR or virus isolation, or in which BTV antibody-specific precolostal antibodies were found, were subjected to further retrospective collection of clinical data.

Differential diagnosis – laboratory testing
Samples from suspected clinical BT cases received for testing at the KVI were routinely tested for an array of pathogens appropriate for...
the clinical signs seen in the animals. All samples were routinely tested for epizootic haemorrhagic disease virus (EHDV) (Yadin and others 2007), foot-and-mouth disease virus (FMDV) (Yadin and others 2007a) and bovine ephemeral fever virus (BEFV) (Yeruham and others 2010).

Animals with conjunctivitis and nasal discharges were tested for sheep-associated malignant catarrhal fever (SA-MCF) (Brenner and others 2002) and ovine herpesvirus type 2 (OvHV-2). Calves showing neurological signs (maladjustment syndrome) were tested for the presence of pathogens that might cause neonatal calf diseases (Brenner and others 2005), and brains from malformed calves were routinely tested for Akabane and Aino viruses by real-time-PCR (Stram and others 2004).

The lungs and internal organs from cows that died showing respiratory signs were tested for respiratory and systemic viral pathogens including parainfluenza virus type 3, bovine viral diarrhoea virus and bovine respiratory syncytial virus (Lynsyansky and others 2009, Friedgut and others 2011). The lungs were also tested for relevant bacterial pathogens such as Mycoplasma atelascens, Mycoplasma bovis and Mannheimia haemolytica.

Cattle exhibiting subcutaneous emphysema were tested for clostridial pathogens.

Results

Outbreaks of BT, associated with severe clinical disease in cattle, occurred on farms across Israel from 2008 to 2010. During the investigations, the presence of BTV RNA was confirmed by RT-PCR in 105 dairy herds and 50 sheep flocks. Laboratory investigations identified seven serotypes of BTV (BTV-2, BTV-4, BTV-5, BTV-8, BTV-15, BTV-16 and BTV-24) and there were many instances of several serotypes being detected on the same farm. In one instance, a cow on one farm exhibited a mixed infection with three distinct serotypes (BTV-4, BTV-8 and BTV-24). A few cases were also confirmed by the detection of precoelostal specific BTV antibodies in sera from aborted fetuses or malformed neonates.

In the dairy farms on which clinical signs of BT were observed, cattle in the late stages of pregnancy and/or immediately postcalving suffered the most severe clinical signs. Approximately 30 per cent of BT-affected animals did not reach their expected milk yield. In addition, several BT-affected farms reported an increased incidence of weak and malformed calves, from which BTV-specific antibodies were detected in precoelostal sera. During the course of the outbreaks of BT in Israel, six distinct clinical ‘syndromes’ were observed in dairy cattle: ‘footrot-like syndrome’, ‘sore nose syndrome’, ‘subcutaneous emphysema syndrome’, ‘red/rough udder syndrome’, ‘bluetongue/epizootic haemorrhage systemic syndrome’ and ‘maladjustment syndrome’.

Footrot-like syndrome

Footrot-like syndrome was seen in a large dairy farm located close to the Israeli-Lebanese border (Fig 2). BTV-4, BTV-8 and BTV-16 were detected in cattle on the farm, and the cattle tested negative for EHDV, FMDV and BEFV.

Adult lactating dairy cattle on the farm suffered from lameness and associated ‘leg problems’. All affected animals were examined by a veterinary surgeon and affected hooves were pared. No subsequent clinical improvement was seen, which suggested that the condition was caused by an infectious agent. Stiff gait and lameness, caused by haemorrhagic lesions within the hoof, were the main clinical signs seen, but other minor manifestations consistent with commonly seen clinical signs of BT or EHDV infection were observed. When the affected hooves were excised, some small necrotic areas were seen, along with acute purulent inflammation of a digit (hoof). Other clinical signs included fever, oedema of the limbs, conjunctivitis and decreased milk yield (by up to 30 per cent). During the outbreak, which lasted for approximately six months, almost one-third of the 250 cows on the farm showed some degree of clinical signs and, during the first three months a 16 per cent case mortality was observed on the farm. Serum neutralisation tests performed on sera from 36 convalescent cows suggested that many of the animals had been exposed to three BTV serotypes, BTV-4, BTV-8 and BTV-16. These three serotypes of BTV were also detected by real-time-PCR in cattle on this farm during 2008/09 (Brenner and others 2010). Two blood samples revealed concurrent dual infections of BTV-4/BTV-8 in one sample and BTV-8/BTV-16 in a second sample. Clinical signs of BT persisted on the farm from August 2008 through to March 2009.

Red/rough udder syndrome

Red/rough udder syndrome related to BTV-8 infection was seen in a large dairy farm (AH) located in the northern Jordan Valley, close to the Golan Mounts (Fig 3). Lactating cattle on the farm exhibited reddening and roughening of the skin of the udder, which resembled dermatophytosis. The signs attributed to BT lasted from the beginning of October to the end of November 2009. Clinical signs included necrosis of the nipple tip, udder discoloration, and oedema and changes of the udder skin that resembled photosensitisation.
previously seen in cattle infected with EHDV-7. To the touch, the affected skin between the teats and the lower part of the udder felt like sandpaper. The affected lactating cows exhibited other characteristic signs of orbivirus infection such as a sharp drop in milk production and marked loss of body weight (Yadin and others 2007b, 2008). Laboratory investigations confirmed the presence of BTV-8 in affected cattle on the farm.

A second dairy farm (TZ) located close to Jerusalem suffered from red/rough udder syndrome, but concurrently a few cows also exhibited clinical signs consistent with BT/EHD systemic syndrome (for clinical description see the section on BT/EHD systemic syndrome below). The clinical signs were seen from June to December 2009. Necrosis of the tip of the teats was noted in both syndromes and five of the 20 diseased animals were culled due to mastitis. Laboratory investigation detected BTV-24 in the affected cows on this farm.

Maladjustment syndrome
Maladjustment syndrome was seen in a dairy farm (PD) located at the northern fringe of the Negev desert (Fig 4). In this farm, a ‘storm’ of newborn calves exhibiting weak calf syndrome (WCS) was recorded, and laboratory investigations confirmed the calves to be infected with BTV-8. No viral or bacterial enteropathogens were detected in the calves and the brains from malformed calves tested negative for Akabane and Aino viruses.

Due to the high percentage of neonatal mortality observed on the farm, instead of WCS, the maladjustment syndrome was used to describe the clinical signs associated with this outbreak. The affected newborn calves were born to primiparous dams during an eight-month period from August 2009 to March 2010. The mortality in this group was 54 per cent (20 of 37) and 14 of the 20 deaths occurred in calves between 12 and 72 hours after birth. The newborn calves appeared normal immediately after birth, with normal posture, good vitality and no respiratory distress; however, after a few hours, the calves exhibited muscle tremors, uncoordinated gait (staggering) and marked respiratory distress that manifested itself as open-mouth breathing, abdominal respiration and lingual protrusion (Fig 4a). The heads of the calves oscillated from side to side, giving the impression of injury to the CNS. Rectal temperatures were 40°C and death ensued a few hours later. Isolation of BTV was attempted only from the 13th defective calf because, up to then, no BT-associated signs had been suspected by the local veterinarian. After a positive PCR result for BTV-8 was obtained from the 13th calf, an additional blood sample was taken from the 12th calf, which was tested retrospectively, and was also found to be BTV-8-positive by PCR. A third intrauterine BTV-8 infection was confirmed in the blood sample taken from the 14th newborn calf, which also exhibited similar signs. Two of the calves exhibiting signs of maladjustment syndrome were subjected to postmortem investigation at the KVI. Both calves presented with extensive muscle haemorrhages, especially of the leg muscles and along the spinal cord (longissimus dorsi) (Fig 4b); however, no microscopic lesions were detected in the brain or in the spinal cord. No relevant enteric or respiratory pathogens were identified in the calves.

Subcutaneous emphysema syndrome
Subcutaneous emphysema syndrome was seen in two large dairy farms (BK and DV) located in the northern fringe of the Negev desert, approximately 10 km north of the town of Beer-Sheba. BTV-4 was isolated from cattle on both farms.

A series of sporadic cases of subcutaneous emphysema were recorded in adult dairy cattle on the farm. The clinical signs attributed
During these outbreaks, a total of 10 cows died and all exhibited similar gaseous subcutaneous lesions that on touch produced typical crepitus sounds. Laboratory investigations confirmed the presence of BTV-4 in affected cattle on both farms. No respiratory distress or lung pathology was reported in the affected cows. As clostridial disease (blackleg) was suspected to be causing the subcutaneous emphysema, muscle biopsies were taken from the affected cows and sent to KVI, however, no Clostridium species were identified in the samples.

**Sore nose syndrome**

Sore nose syndrome was seen in a dairy farm (DO) located approximately 10 km west of BK (Fig 5). BTV-4 was isolated from cattle on the farm. The cattle tested negative for EHDV, FMDV, BEFV, SA-MCF and OvHV-2.

Cattle on the farm exhibited inflammatory lesions of the nostrils. This syndrome was documented for more than one year, from August 2006 to November 2009. In total, 10 cases of sore nose syndrome were reported, mainly in primiparous lactating cows, immediately after they had exhibited a sudden sharp drop in milk production. Limb oedema and/or udder oedema was also noted, and in addition, two cases of subcutaneous emphysema were reported.

**Bluetongue/epizootic haemorrhagic systemic syndrome**

Bluetongue/epizootic haemorrhagic systemic syndrome (BT/EHD systemic syndrome) was seen in two adjacent dairy farms (KL and AL) located in the southern part of the Jordan Valley River, near the Dead Sea shore, where EHDV was first detected in September 2006 (Yadin and others 2007b, 2008) (Fig 6). During 2006 to 2009, BTV-24 was isolated from cattle in these two dairy farms. The cattle tested negative for EHDV, FMDV and BEFV.

During the EHDV outbreak in 2006, the disease was categorised as ‘BTV-like disease’, because at that time, EHD had never previously been recorded in cattle in Israel. The striking similarity of the clinical manifestations seen in cattle infected with BTV with the clinical signs seen in cattle during the 2006 outbreak of EHD in Israel (Yadin and others 2008) has led the authors to define this new syndrome.

BT/EHD systemic syndrome was reported in cattle on the two farms from August 2008 through to November 2009. In these two herds, five primiparous heifers out of 500 milking cows died and typical signs of EHD were reported in many of the cattle. The first signs included a sharp drop in milk production, which was accompanied by weight loss and loss of appetite. Other typical clinical manifestations observed included fever, stiff gait, reddening of the nasal and lip mucosa, swelling of the tongue, buccal erosions, udder discoloration, conjunctival hyperaemia and palpebral oedema, and intramural hoof haemorrhages. In addition, other non-specific manifestations were observed, which included recumbency, reduced ruminations, submandibular oedema and restlessness.

**Discussion**

In the period from 2006 to 2011, multiple strains of BTV have been identified to be circulating in Israel. On some farms, up to three distinct serotypes of BTV have been identified, and in some cases up to three serotypes have been identified to be circulating in the same animal. Some herds in Israel experienced two or even three years during which the cattle exhibited several different BT-related clinical manifestations, which suggests exposure to several different BTV serotypes. In fact, BTV-8, which was identical to the BTV-8 strain recently found to be circulating in Europe, was detected in dairy cattle farms that had previously been infected with BTV-4. In a sheep farm, BTV-4 ‘replaced’ BTV-8, and in a goat in which BTV-16 was detected in 2009, BTV-8 was detected in 2010. These results indicate that livestock in Israel were being infected with multiple serotypes either at the same time or over a relatively short period of time.

The reason why so many new outbreaks of BT, caused by apparently novel serotypes of BTV, have been identified in Israel from 2008 to 2010 is currently unknown. In the past two years, there has been an unprecedented spread of at least seven BTV serotypes across Israel and probably into the Middle East region. Clinical signs have been seen for the first time in Israeli cattle and a field strain (BTV-8) has been proved for the first time to have the ability to cross the placenta and infect calves in utero. No vaccination has been carried out in response to these outbreaks, so these novel viral strains have been allowed to spread freely within the region. This has provided multiple opportunities for the exchange/reassortment of genome segments,
as seen before (Batten and others 2008, Maan and others 2010). It is therefore highly likely, as so many different BTVs are co-circulating at the same time, that reassortment of these circulating viruses will have occurred in the field, resulting in the appearance of new viruses with unique phenotypes. This could also be the reason for the wide array of clinical signs/syndromes that are currently being observed in Israel, and are described in this paper.

The clinical signs associated with the ‘syndromes’ that are described in this paper could be caused by an array of different pathogens. In order to confirm the potential role of BTV as the primary causal agent, samples were routinely tested for pathogens appropriate to the clinical signs seen in each ‘syndrome’. All samples were routinely tested for EHV-4, FMDV and BFEV. Animals with conjunctivitis and nasal discharges were tested for SA-MCF and OvHV-2. Calves showing neurological signs (maladjustment syndrome) were tested for Akabane and Aino viruses, and cows exhibiting subcutaneous emphysema were tested for clostridial infection. In all cases, and for all the syndromes, BTV was the only pathogen that was definitively identified. In BTV-negative cases, BTV serotype testing was performed, and in some cases, other field strains, thus enabling other field strains of BTV to cross the placenta and infect calves. In the present study, however, other pathogens were not identified by laboratory testing, were causing some of the clinical signs seen, and that BTV may have predisposed the animals to these infections.

In Israel, calf malformations were formerly thought to be associated only with Simbu serogroup infection (Markusfeld and others 2000, Batten and others 2004a, b) and were regularly reported to occur in farms where BTV-8 signs have not been reported. The BTV-8 strain from Europe, which is the same as the BTV-8 strain currently circulating in Israel, is currently considered to be unique as a field strain in its ability to cross the placenta and infect unborne calves (Darpe1 and others 2009). This was thought to only laboratory-adapted strains of BTV, such as the live attenuated vaccine strains, were capable of transplacental transmission. Since the transplacental strain of BTV-8 in Israel, probably through the importation of infected animals from Europe (Brenner and others 2010), many cases of transplacental transmission induced by BTV-8 have been reported and confirmed in cattle in Israel. Up to now, despite identifying up to seven serotypes of BTV circulating in cattle in Israel, only BTV-8 has been proven to have the ability to cross the placenta and infect calves. In the present study, there is some indirect evidence for transplacental transmission of BTV-8 that has been transmitted to calves from the mother. In calves born from infected dams, there was no evidence for BTV-8 in the calves at this time, however, other field strains of BTV may be possible, however, that other pathogens, not identified by laboratory testing, were causing some of the clinical signs seen, and that BTV may have predisposed the animals to these infections.

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