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Short Communication

Description of the first acute bovine diarrhea virus-2 outbreak in Israel

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ABSTRACT

This is the first report of an acute and fatal outbreak of bovine diarrhea virus (BVDV)-2 infection in Israel. The clinical presentation varied with the age of the affected animals with a bovine–respiratory–complex–like syndrome in young stock, and diarrhea and dysentery only in the lactating stock. Enteritis first appeared in one shed of post-parturient cows; it spread for 6 weeks, until at least 30% of the lactating stock contracted enteritis or dysentery. At the same time, dairy calves aged 10–90 days exhibited severe respiratory disease. Of 79 animals that died, 13/350 (3.7%) were adult lactating cows, and 66/1100 (6%) were young feedlot calves. Phylogenetic analysis of the isolated virus revealed a 95% identity with the corresponding genome parts of various BVDV type 2 sequences. The route of introduction of BVDV-2 into Israel could not be elucidated.

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Bovine viral diarrhea virus (BVDV), a member of the genus *Pesivirus*, comprises two distinct species, BVDV-1 and BVDV-2 (Heinz et al., 2000). Post-natal infection elicits a wide range of clinical manifestations and pathological conditions. Additionally, infection in the first trimester of pregnancy can result in persistent infection (PI) of the offspring, which is often unnoticed (Radostits et al., 2007).

In the non-pregnant animal, exposure to BVDV-1 often elicits no or only mild clinical signs, but may cause susceptibility to other pathogens, most of which have a tropism to the respiratory tract (Baker, 1995). Severe courses of BVDV infection in adult animals, characterized by thrombocytopenia and high mortality (hemorrhagic syndrome) have been linked exclusively to BVDV-2 infection (Ridpath et al., 1994), but Lunardi et al. (2008) recently described a wild-type BVDV subtype 1b that caused an acute outbreak in beef cattle in Brazil. It elicited similar clinical signs to BVDV-2 infection, from which it could be distinguished only by molecular analysis.

Here we present the first report of acute BVDV-2 infection in Israel. The progress of the disease was severe as expected, but predominantly with enteritis and respiratory symptoms rather than a hemorrhagic syndrome. The location was a BVD-unvaccinated mixed farm which housed approximately 350 lactating Israeli Holstein–Friesian cows and 300 heifers; it also raised male calves that were acquired at 1 week of age from five other dairy herds, and had

a feedlot of 1100 mixed-breed calves that were bought from several sources.

In March 2008, the first noticeable problems, namely, fever, lack of appetite, and agalactia, were seen. Enteritis first appeared in one shed of post-parturient cows, and by early-September 2008, at least 30% of the lactating stock contracted enteritis or dysentery, and dairy calves aged 10–90 days exhibited severe respiratory discomfort. The lactating stock, were located less than 50 m from the gravid dry cows, among which clinical signs were first noted, and young stock (aged 1–3 months) were 50 m from the gravid dry cows and 100 m from the lactating cows. The feedlot was located 300 m outside the main dairy premises, with which it shared no facilities, but feed was distributed from there throughout the farm. Calves were moved to the feedlot at 90 days of age and these calves (aged 3–10 months) were less affected, if at all.

Each affected lactating cow exhibited temperatures of 41–41.5 °C for 4–5 days accompanied by diarrhea. Overall, dysentery was present in the adult animal stock for approximately 3 months. The lactating cows were first diagnosed via the milking records, when milk yield decreased. Tentative treatment of the young stock that exhibited respiratory distress achieved no clinical improvement, in spite of routine administration of 'preventive respiratory' doses of tetracycline (Aurofac Aureomycin, Alpharma) before the outbreak, and of therapeutic doses of enrofloxacin (Baytril, Bayer) during prevalence of the respiratory symptoms. This wave killed 79 animals, comprising 13 adult cows out of 120 affected lactating cows (i.e. a case mortality rate of approximately 0.09), and 66 calves under than 90 days of age (i.e., 3.7 and 6% of the respective populations).

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Five heifers had tested negative for antibodies against BVDV in November 2007, indicating that no persistently infected (PI) animals were present, the lack of clinical BVD-infection-related manifestations suggested there was no latent or acute infection in this herd (Lindberg and Alenius, 1999). BVDV virus was first isolated on 17 July 2008 at the Kimron Veterinary Institute (KVI), during a pathological investigation of colon-sections of adult animals that had died. Previously, 60 young calves aged 10–90 days had succumbed and the fresh carcass of a young calf was taken to KVI for post mortem examination. The carcass exhibited severe pneumonia; 70% of the lung parenchyma was severely affected, and consolidation, color changes and micro-abscess were scattered on much of the lung mass. No other changes were noted in the internal organs, but the stomach and the fore-stomachs were empty. Histological examination revealed multifocal necrosis, and massive infiltration of alveoli and bronchi, neutrophils and histiocytosis. Eleven additional lungs from calves aged 8 to 12 weeks, also had severe lung affections (Table 1).

Additionally, part of the intestine of the dead cow was available and exhibited discernable mucosal hemorrhages ('zebra stripes'). The tissue was suitable for virus isolation and identification, but not for histopathological examination. The attending veterinarian had reported that all five adult cows examined had presented with a congested intestinal tract, bloody feces and intestinal hemorrhages.

Two commercial BVDV antigen (BVDVAg) detection kits, the HERDCHEK BVDV Ag/Leukocytes, and the HERDCHEK BVDV Ag/serum plus (IDEXX Laboratories), were used, and virus was isolated using Madin–Darby Bovine Kidney Cells (MDBK). Cell-culture supernatants from infected cells were used for reverse transcription polymerase chain reaction (RT-PCR) and subsequent genotyping.

Twelve blood samples and the intestinal section (Table 2) yielded BVDV RNA, which was confirmed by RT-PCR. RNA was extracted from cell-culture supernatants with the QIAamp Viral RNA Kit (Qiagen). The following primers were used: B3 5'-GGT-AGC-AAC-AGT-GGT-GAG-3'/B4 5'-GTA-GCA-ATA-CAG-TGG-GCC-3' and B5 5'-ACT-AGC-GGT-AGC-AGT-GAG-3'/B6 5'-CTA-GCG-GAA-TAG-CAG-GTC-3', for BVDV-Typ I and BVDV-Typ II, respectively. RT-PCR revealed a typical 220-bp band with the BVDV-2-specific primers but not with the BVDV-1-specific primers (Letellier et al., 1999; Vilcek et al., 2003). We used the following sequence: TAG-CAGTGAGTTCATTGGATGGCCGAATCCCTGAGTACAGGGAAGTCGTCA TGGTTCGACACTCCATCAGTTGAGGAGTCTCGAGATGCCATCATAGGT GAAAGCGCCATTCGTGGTGCTATGGACACAGCCTGATAGGGTGTAGÓ TAGCAGTGAGTTCA TTGGATGGCCGAATC, for the BLAST nucleotide search and it revealed a 98% nucleotide identity with the following BVDV-2 strains: EU224225.1, AY379543.1, AY379544.1, AY379545.1, and AJ304381.1. The products were sequenced by Eurofins MWG Operon, Martinsried, Germany.

Table 1

Summary of BVDV antibody and BVD antigen results indicating the probable link between BVDV-2 infection and the outbreak.

Stock tested	Number of animals found BVDVAb positive (number of submitted samples)	Number of animals found BVDVAg positive (number of submitted samples)
Young animals	Not done	9 (12)
Lactating cows		
Diseased	2 (4)	13 (13) ^a
Convalescent	5 (7)	0 (5)
Apparently healthy	0 (3)	0 (3)

^a Twelve blood samples and one intestinal-section sample. Differential laboratory diagnoses included bovine ephemeral fever, epizootic hemorrhagic disease, blue-tongue, rinderpest, para-influenza-3, bovine respiratory syncytial virus, which were all negative; only one sample was positive for *Mycoplasma bovirhinitis*.

Table 2

Timetable of tentative clinical diagnoses advanced after the confirmation of the first BVDVAg positive sample (17 July 2008 onwards).

Date (2008)	Tentative diagnoses and tests requested (for adult or young animal stock)	Laboratory test outcome
17 July	Pneumonia (young calf)	PIV3, BRSV, <i>Mycoplasma</i> spp. negative. BVDVAg positive
	16 EDTA blood and 14 sera of diseased and convalescent (adult) animals (see text)	7/7 BVDVAg positive (adult cows) and 7/7 thrombocytopenia 5/7 BVDVAb positive convalescent adult cows
17 June, 7 July, 17 August ^a	Clinical examination of five lactating cows BEF, EHD, indigestion, respiratory distress (five lactating cows) ^a	Marked cyanosis and diarrhea/dysentery 5/5 BVDVAg positive ^a
28 July, 8 August, 8 September	Organs from (seven young calves)	4/7 BVDVAg positive

^a These samples remained from the previous tests (Table 1), and were retested this time for BVDVAg, only after the KVI team suspected BVDV infection.

Fourteen sera from adult animals were tested with the commercial BVD antibody (BVDV-Ab) SVANOVIR kit (Svanova Biotech). Sera were taken from seven convalescent, four clinically affected, and three apparently healthy cows. Five of the seven convalescent animals were BVDV seropositive, compared with only 2/4 diseased animals and 0/3 apparently healthy cows. Interestingly, one of the seropositive cows had tested sero-negative a month previously, which led to the present diagnosis of BVDV seroconversion. All other adult cows had been tested only once.

The relevant hematological outcomes (ranges) of seven blood samples of cows with pale mucosa were: RBC $5.2\text{--}6.85 \times 10^6$ (reference, $7.4\text{--}11.6 \times 10^6$); hemoglobin 7.4–7.8% (9.8–15.3%); hematocrit (packed cell volume) 21.0–28.9% (reference, 25.8–40.1%); leukocytes $0.82\text{--}5.8 \times 10^3$ (reference, $6.2\text{--}13.6 \times 10^3$); and thrombocytes; $45\text{--}384 \times 10^3$ (reference, $412\text{--}1003 \times 10^3$).

BVDV infection is an important infectious disease of cattle worldwide. Recognized syndromes associated with BVDV infection include subclinical infection, reproductive disturbances, and gastrointestinal and respiratory diseases. Fatal mucosal disease has been known as an acute syndrome characterized by gastrointestinal lesions, which occur only in PI animals (Ellis et al., 1998; Sreerama et al., 2008). A specific form (the hemorrhagic syndrome) is predominantly linked to BVDV-2 infection. Since no BVDV type 2 hemorrhagic infections had been observed in Israel before the present episode, acute severe BVDV-1 infection was suspected as the cause of respiratory and enteric symptoms in this affected herd.

Previously, only BVDV-1 has been found in Israel. We collected and analyzed samples taken during the years 1996–2007 in various locations, and sequenced and analyzed them in order to characterize and map the isolates. Further analyses revealed that the isolates could be subtyped into BVDV type 1b (unpublished data).

Viruses tend to exacerbate other latent bacterial and viral pathogen infections. *Mycoplasma bovirhinitis*, an apathogenic bacterium, was isolated from the lung of the dead calf, but is unlikely to have contributed to the severe pneumonia or dysentery manifestations. BRSV or other latent viruses were not found in any of the seven lungs submitted. Although any strains of bovine respiratory coronaviruses could be involved in winter dysentery (WD), exacerbation of such viruses is very unlikely in this case, since the WD syndrome is not present in Israel. Therefore, these findings could not explain the marked difference between clinical signs shown at different ages observed in this descriptive study of BVDV-2.

Table 3

Average milk yield and conception rates in lactating cows on the farm before, during and after the BVD type 2 outbreak.

Year	Average milk yield per cow (kg)	Conception (%)		Culls (n=)
		Primiparous	Multiparous	
2007	11,639	45.4	37.0	78
2008	11,595	41.7	27.0	109
2009	11,198	36.4	32.0	112

To our knowledge this is the first description of a fatal outbreak of acute BVD related to BVDV-2 infection in Israel. This case was characterized by a clinical dichotomy: a bovine–respiratory–complex–like syndrome (BRCS) was noted only in young stock, whereas diarrhea or dysentery was observed only in the lactating stock. Remarkably, the clinical signs presented by the adult animals did not resemble published descriptions of severe infection with BVDV genotype 2 (Carman et al., 1998; Flores et al., 2000; Odeón et al., 2003). For 15 months 45/615 (7.3%) calves aged 3–5 weeks old tested were categorized as PI. The last ones detected were from May (4), August (1), September (1) and October (1), 2009. In addition, to the immediate losses of animals during the acute stage of the outbreak, the productive and reproductive damages are shown in Table 3.

All of the herds from which young male calves were purchased claimed not to be BVD positive. This claim had been based on periodic voluntary serial serological sampling of their lactating and young stocks (Lindberg and Alenius, 1999). Only after about a year following diagnosis of the BVDV-2 acute infection in the studied herd, BVDV-2 was found in the neonate farm of one of the six suppliers. Diarrhea and pneumonia were the only clinical signs noted at this source and no other signs were reported in the lactating stock.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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