

## Concurrent occurrence of a bovine viral diarrhoea virus type-1 (BVDV-1) infection and *Trueperella pyogenes* bronchopneumonia in a calf

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**Citation:** Eroksuz Y, Abayli H, Yerlikaya Z, Incili CA, Karabulut B, Eroksuz H (2021): Concurrent occurrence of a bovine viral diarrhoea virus type-1 (BVDV-1) infection and *Trueperella pyogenes* bronchopneumonia in a calf. Vet Med-Czech 66, 167–171.

**Abstract:** The concurrent occurrence of a bovine viral diarrhoea virus type-1 infection and necrotising-suppurative bronchopneumonia due to *Trueperella pyogenes* was diagnosed in a 4-month-old male calf. The pulmonary lesions were characterised by necro-suppurative bronchopneumonia with intralesional gram-positive microorganisms. The reverse transcription polymerase chain reaction (RT-PCR) and matrix-assisted laser desorption ionisation time-of-flight mass spectrometry analysis (MALDI-TOF MS) also indicated the presence of BVDV type-1 and *Trueperella pyogenes* agents, respectively. A positive immunoreaction to the BVDV was present in the vascular walls in the lungs, hepatocytes, lymphoid cells in the spleen and lymph nodes, and neurons in the brain. With this case study, the two infections were, to the best of our knowledge, reported concurrently for the first time. It can be assumed that a subclinical BVDV infection might contribute to the occurrence of *Trueperella pyogenes* infections in calves.

**Keywords:** animal diseases; bovine; PCR; ruminant

Bovine viral diarrhoea virus (BVDV) is categorised in the *Pestivirus* genus within the *Flaviviridae* family. A BVDV infection results in various syndromes depending on the virulence, immune status of the host, whether there is a pregnancy, and the stage of the pregnancy. Amongst these syndromes, BVDV often causes a subclinical infection characterised by a mild fever, leukopenia and immunosuppression in immunocompetent, seronegative and non-pregnant animals (Caswett and Williams 2007). As BVDV has a predilection to lymphoid tissues, a viral replication might cause follicular hypoplasia and inhibit the normal local and systemic immune functions, and immune defence mecha-

nisms and enhance the disease processes induced by respiratory and other pathogens (Caswett and Williams 2007; Radostits et al. 2007).

Most frequently, BVDV infections have been reported together with other viral and bacterial infections including bovine herpesvirus 1 (BHV-1), bovine parainfluenza virus 3 (BPI-V-3), bovine respiratory syncytial virus (BRSV), bovine papular stomatitis virus (BPSV), bovine rotavirus (BRV), bovine coronavirus (BCV), *Mannheimia haemolytica*, *Pasteurella multocida*, *Streptobacillus actinoides*, *Salmonella typhimurium* and *Escherichia coli* (Jeffery and Hogg 1988; Jensen and Schultz 1991; Fulton et al. 2000; Wang et al. 2019).

*Trueperella pyogenes* is a widely known gram-positive, rod-shaped, non-spore-forming bacterium causing opportunistic pyogenic infections in livestock. It is a part of the microflora of skin and mucous membranes of the upper respiratory, gastrointestinal and urogenital tracts in farm animals (Rzewuska et al. 2019). However, this microorganism causes a necro-suppurative inflammatory response and abscess formation in a variety of organs as it is present on the mucosal surfaces and skin. Moreover, *Trueperella pyogenes* may cause a variety of other suppurative infections and abscess formations in the lungs, brain, kidneys and lymph nodes in cattle (Riberio et al. 2015). Bronchopneumonia due to *Trueperella pyogenes* was reported earlier in calves, and other farm animals with morphological changes. However, the concurrent occurrence of infections caused by *Trueperella pyogenes* and BVDV have not been recorded.

In the present report, the concurrent occurrence of a BVDV-1 infection and necrotising and suppurative bronchopneumonia due to *Trueperella pyogenes* has been described in a 4-month-old calf.

## Case description

A 4-month-old, male Simmental calf was brought to the Animal Hospital of Firat University Veterinary School of Medicine. The animal was from a family herd containing 6 cows and 2 calves. The calf gradually became dull and lethargic, dehydrated, showing inappetence, and weakened despite antibiotic and supportive care for 2 days. The animal was found dead in the morning of day 3. At necropsy, except for the lungs, there were no other prominent gross lesions. In the lungs, the cranio-ventral lobes (40%) were mottled dark red, and firm on palpation. On the cut surfaces, there was multifocal to coalescing white focal nodular lesions in the affected pulmonary parenchyma.

The microscopical changes included multifocally distributed suppurative and necrotic-desquamative bronchopneumonia, diffuse alveolar histiocytosis and rare multinucleated histiocytes and intraleisional gram-positive microorganisms in the lungs (Figure 1A–D).

An avidin-biotin complex method was performed as previously described by Hsu et al. (1981). A BVDV antigen was detected in the cerebral cortical neurons, bronchial epithelium, alveolar cells

and alveolar macrophages, hepatocytes (Figure 1E), renal tubular cells and myocardial Purkinje cells.

The RNA extraction from the formalin-fixed paraffin embedded (FFPE) brain, lung and kidney tissue sections was performed manually. First, the samples were deparaffinised by the addition of 1.0 ml xylene followed by incubation for 7 min at 50 °C, and centrifugation for 5 min at a maximum speed (18 800 g). Secondly, the supernatant was discarded, and the pellet was washed three times with 1.0 ml of absolute ethanol. Finally, the pellet was lysed with 1 mg of a proteinase K added TRIzol reagent. After 1 h of incubation at 56 °C, the TRIzol reagent (Thermo Fisher, Waltham, MA, USA) instructions were followed. The RNA pellet was resuspended with 50 µl of RNase free water.

The amplification of the RNA by reverse transcription polymerase chain reaction (RT-PCR) performed with the One-step RT-PCR kit (Qiagen, Hilden, Germany). The kit's instructions were followed. The amplification conditions were 50 °C for 30 min and 95 °C for 15 min, followed by 35 cycles of denaturation (94 °C, 30 s), annealing (52 °C, 60 s) and extension (72 °C, 60 s). The B3B4 and B5B6 primer pairs were used to detect the BVDV type 1 and 2 RNA, respectively (Letellier et al. 1999). The RT-PCR products were electrophoresed in a 1.5% agarose gel and were analysed (Figure 2).

The unidirectional sequence analysis of the amplicons obtained at the end of the RT-PCR was performed by MacroGen Inc. (Amsterdam, The Netherlands). At this stage, an ABI3730XL Sanger sequencing device (Applied Biosystems, Foster City, CA, USA) and Big Dye Terminator v3.1 cycle were used. BVDV-1 was confirmed by the nucleotide BLAST software (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The sequence data of the BVDV-1 strain in this study was deposited in the GenBank database with the accession number MT050031.

For the isolation of the bacteria, 5.0% sheep blood was added to the blood agar. Incubation was performed at 37 °C for 24–48 h in an aerobic and a 10% CO<sub>2</sub> environment. After the gram staining and motion examination were performed for the suspected colonies, the biochemical catalase and oxidase activities were measured. Beta haemolytic, small, white-opaque colonies were detected at the end of the incubation, gram-positive and pleomorphic rods were observed. The motion examination revealed that the bacteria were inactive. The catalase and oxidase reactions were negative.



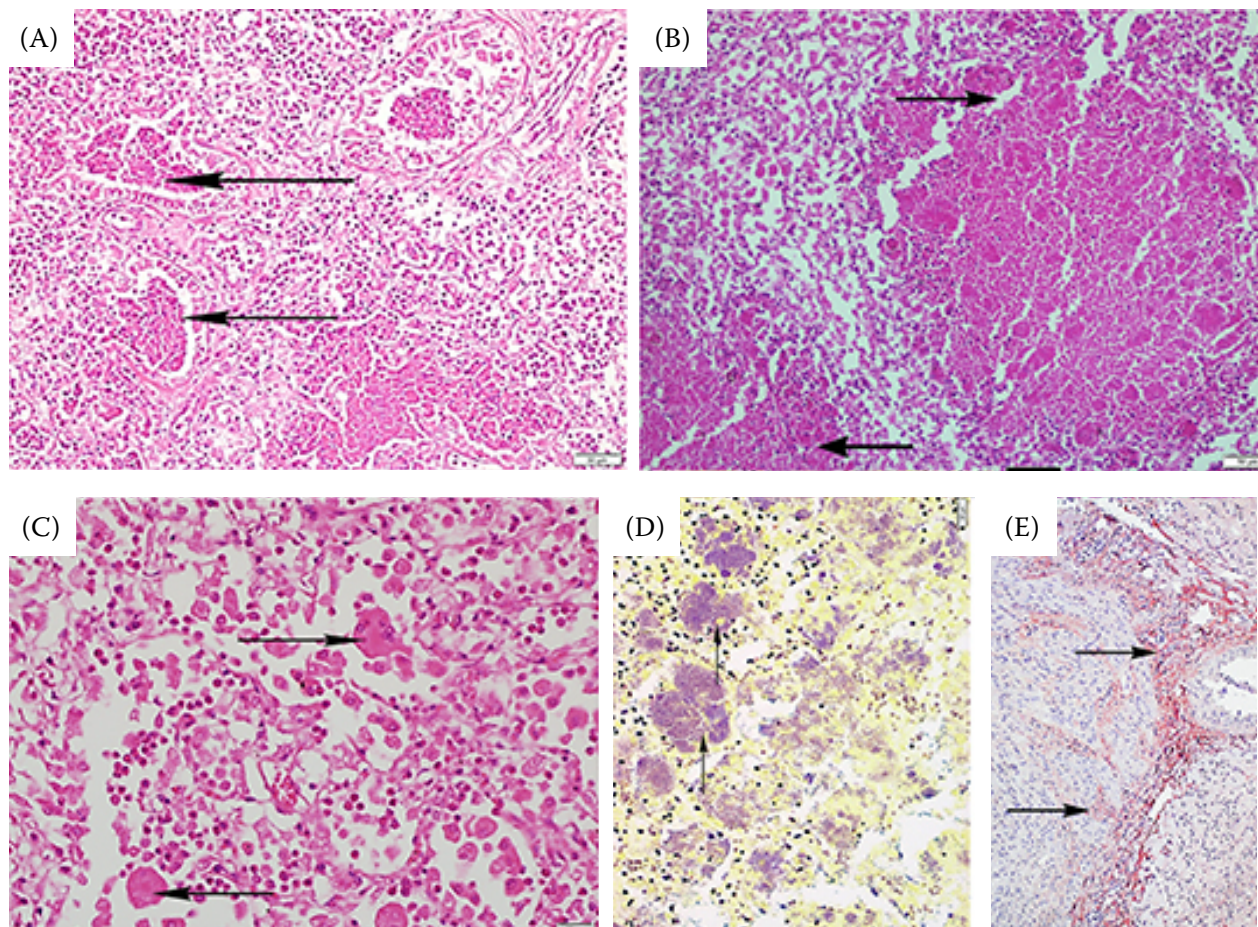


Figure 1. (A–B) Severe multifocal necrotising bronchopneumonia characterised by necrotic bronchiolitis and necro-suppurative foci (arrows) in the pulmonary parenchyma (H&E). (C) Diffuse alveolar histiocytosis and multinucleated cells (arrows) (H&E). (D) Clumps of gram-positive microorganisms (arrows) in the necrotic focus (B-B gram stain). (E) Positive immunoreaction in the hepatocytes and periportal connective tissue (arrows)

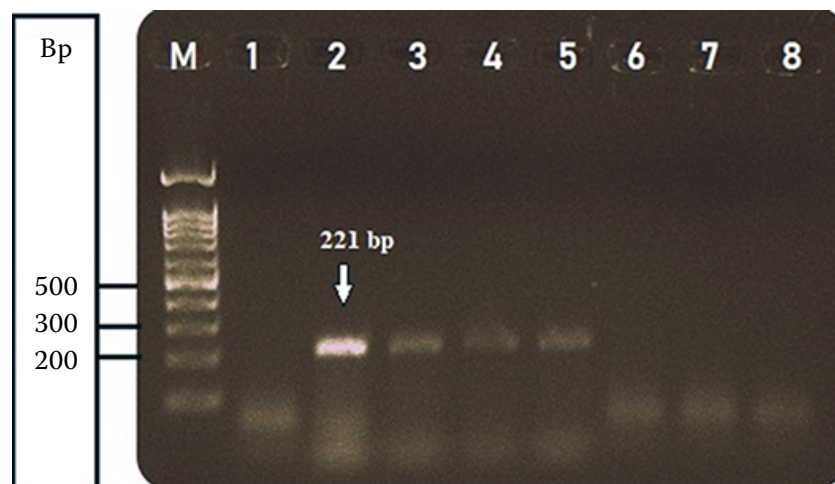


Figure 2. Electrophoresis of the PCR products of the BVDV genome obtained by RT-PCR on the 1.5% agarose gel electrophoresis. M; 100 bp DNA ladder (Cleaver Scientific, Warwickshire, England), lane 1; negative control, lane 2; positive control [NADL (National Animal Disease Laboratory; Ames, Iowa, USA) strain], lane 3–4–5; BVDV type-1 positive clinical samples (lung, brain and kidney, respectively), lane 6–7–8; BVDV type-2 negative clinical samples (lung, brain and kidney, respectively). Sequencing kit (Applied Biosystems, Foster City, CA, USA) was used

The MALDI-TOF MS based bioMérieux VITEK MS (database v2.0) was defined as *Trueperella pyogenes*. In order to verify the result of this identification; a partial 16S rDNA sequence was performed using the primers p8FPL 5' D AGT TTG ATC ATG GCT CAG-3' T and p806R 5A-GGA CTA CCA GGG TAT CTA AT-3' GC. Approximately, 1 200 nucleotides were analysed and the isolate was identified as *Trueperella pyogenes* with 99.0% similarity by using the MALDI-TOF MS.

## DISCUSSION AND CONCLUSION

Over the last decades, *Trueperella pyogenes* has been reported as the cause of different clinical signs in cattle (Radostits et al. 2007). The major occurrence of mastitis, pneumonia, lymphadenitis, abscesses, and miscellaneous other less frequent infections among livestock are caused by septicaemia, encephalitis, pyometra, prostatitis, orchitis, seminal vesiculitis, pericarditis, omphalitis, and interdigital phlegmon (Riberio et al. 2015). However, chronic suppurative pneumonia is also a common clinical manifestation of *Trueperella pyogenes* infections among livestock.

There are no specific control measures to prevent *Trueperella pyogenes* infections in domestic animals including vaccination attempts. *Trueperella pyogenes* might be a primary pathogen or cause a concurrent infection due to the immunosuppression caused by starvation, cold temperature, long transportation, etc. (Riberio et al. 2015). However, a BVDV infection has not been reported as a predisposing factor in literature and classical veterinary textbooks.

The present report documented the dual infection of BVDV-1 and *Trueperella pyogenes*. The immunosuppression caused by BVDV is explained by the decreased interferon production, lymphocytic function, monocytic proliferation and chemotaxis (Caswett and Williams 2007). It also negatively affects the antibody production and neutrophilic functions. These changes are temporary in acute cases, but more permanent in chronically infected animals and those infected with a mucosal disease.

BVDV often leads to a subclinical infection in immune competent, seronegative and non-pregnant animals. A mild fever, leukopenia and special neutralising antibodies develop in 70–90% of BVDV infected animals. After 5–7 days of an incubation

period, a fever, leukopenia and viremia occur in animals (Caswett and Williams 2007).

BVDV has important economic impact on the extensive animal industry. However, the economic impact or cost of BVDV infections is entirely unknown due to the unpredictable sickness behaviour. The economic components of the disease due to the immunosuppression, mucosal disease, abortions, mummification, thrombocytopenic haemorrhage, different congenital malformations according to the stage of pregnancy, decrease in weight gain and milk yield, and the cost of disease prevention and eradication programmes have a lasting effect (Radostits et al. 2007).

Overall, the present report documented the dual infection of BVDV-1 and *Trueperella pyogenes*. A BVDV infection may contribute to the occurrence of a *Trueperella pyogenes* infection in calves due to its immunosuppressive effects.

## Conflict of interest

The authors declare no conflict of interest.

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Received: May 28, 2020

Accepted: November 10, 2020