



Efficient BVD control on large industrial dairy farms infected with different subtypes of Pestivirus A strains

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DOBOS, A., K. SZIGETI, L. CUBAS-GAONA, H. SASVÁRI, M. SAMARDŽIJA, M. KOVAČIĆ, I. KISS: Efficient BVDV control on large industrial dairy farms infected with different subtypes of Pestivirus A strains. *Vet. arhiv* 95, 149-156, 2025.

ABSTRACT

Bovine viral diarrhea (BVD) is a major economically significant disease condition of cattle, occurring worldwide and caused by Pestivirus A, B, and rarely D viruses. Infection by the virus has a peculiar epidemiology that involves immunotolerant, persistently infected (PI) calves, which spread the virus lifelong in high amounts, and therefore finding and eliminating them is the cornerstone of most eradication programs, aided by provision of sufficient immune status for the dams to prevent the emergence of PI offspring. We demonstrate the efficient control of the prevailing Pestivirus A subtypes 1b, 1d, and 1f, in various Hungarian industrial dairy farms, by following the principles of bovine viral diarrhea virus (BVDV) eradication, such as screening the herds for the presence of and removal of PI animals, proper vaccination, and monitoring the BVDV status of the herds by laboratory testing. The tested partial Npro coding sequences indicated the presence of herd specific strains in each farm. The results confirmed that vaccination is a powerful tool for eradication of BVDV even if the live vaccine strain is heterologous compared to the field strains circulating on the affected farms.

Key words: dairy cattle; BVD; control; Npro sequence

Introduction

Bovine viral diarrhea (BVD) is a prevalent infectious disease of cattle. It causes a variety of clinical manifestations, such as abortion, respiratory and intestinal disease, reproductive failures, and immunosuppression, which compromise the

performance of the herd and an adequate response to immunization, and makes them more susceptible to concurrent infections ([BROWNLIE, 1985](#); [BAKER, 1995](#); [MCGOWAN and KIRKLAND, 1995](#); [HOUE, 2003](#); [WALZ et al., 2020](#)).

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BVD occurs world-wide and is common across most of Europe ([SCHARNBOCK et al., 2018](#)). The causative virus, Pestivirus A, exists as two biotypes, non-cytopathic (ncp) and cytopathic (cp) viruses, in relation to their behavior in inoculated cell cultures. The ncp form is considered the “genuine” variant, while the cp viruses arise from them by genetic rearrangements, such as mutations, gene duplications, and homologous or heterologous recombinations ([NETTLETON and ENTRICAN, 1995](#)).

A cornerstone of the epidemiology of BVD is the development of persistently infected (PI) and immunotolerant calves, as a consequence of the infection of susceptible dams during the first trimester of gestation. Although most die before reaching maturity, PI animals might be unrecognized on a clinical basis, and act as “virus factories”, spreading the harbored virus in huge quantities to their mates. Therefore, the key for most BVD control programs is to find and eliminate PI animals and identify so-called Trojan dams, that are pregnant with a PI calf, while themselves being negative for BVDV ([LINDBERG and ALENIUS, 1999](#); [REARDON et al., 2018](#)). Vaccination of susceptible animals is an appropriate tool to control the disease by reducing clinical signs, and, importantly, by preventing the emergence of PI calves ([MOENNIG et al., 2005](#)). Eradication of BVDV without using vaccines was launched in 1993–1994 in Denmark, Finland, Norway and Sweden, and was known as the Scandinavian model ([MOENNIG et al., 2005](#)). These programs have been successful, and these Scandinavian countries are currently either free, or almost free from BVDV ([BITSCH and RONSHOLT, 1995](#); [STAHL and ALENIUS, 2012](#)). According to the latest study, the true herd-level virus prevalence was found to be 12.4% in Hungary ([SZABARA et al., 2016](#)). There is no mandatory national BVD eradication program in Hungary but most large, industrial dairy farms have initiated voluntary eradication programs of the disease ([KOVAGO et al., 2015](#)). Recently we demonstrated that subtypes b, d, and f of Pestivirus A (BVDV-1) strains occur on Hungarian dairy farms, and further, that a subtype a vaccine induced neutralizing antibodies

that cross-reacted with these isolates beyond the established protective limit ([KISS et al., 2022](#)). In order to confirm the laboratory findings, we followed up the BVDV status of three large industrial herds, not vaccinating against BVDV. All three farms were affected by reproductive and respiratory diseases, with BVDV suspected in the background, and therefore, they decided to establish their BVDV status and take the necessary steps to control the infection. The methodology applied was based on testing bulk serum and milk samples by antibody ELISA and virus detecting qPCR, which has already proven useful for PI animal screening ([ZIMMER et al., 2002a](#)).

Materials and methods

Three industrial dairy farms embarked on the control of BVDV, starting in 2020-2021 as listed in Table 1.

The schedule of samplings is shown in Fig. 1-3, indicating the type/number of samples and tests performed, and, importantly, the occasion and number of PI animals identified, and the time points when vaccination was introduced. In a few cases, nasal swab and lymph node samples were also submitted for investigation.

The molecular and serological tests used have been described earlier ([KISS et al., 2022](#)). Briefly, the screening qPCR was the commercially available EXOONE BVDV-BDV (EXOPRUM100) kit (EXOPOL, Spain) and the Npro nucleotide sequences were obtained according to [BOOTH et al. \(2013\)](#). Serological investigations were carried out using the IDEXX BVDV Total Ab ELISA kit (IDEXX, USA).

The vaccination scheme was as follows: all the cows were immunized with a live vaccine (Mucosiffa[®], containing the subtype 1a Oregon C24V strain; CEVA-Sante Animale, Libourne, France) according to the manufacturer’s instructions. All the calves were vaccinated at 2-3 months of age and after 5-6 months of age. An annual booster has given a minimum of one month before breeding. Reportedly, the vaccine induced efficient fetal protection in all vaccinated animals ([MEYER et al., 2008](#); [MEYER et al., 2012](#)).

Table 1. The farms participating in the study and their major parameters

	Farm A	Farm B	Farm C
Herd size	630 dairy cows; 370 heifers	980 dairy cows 620 heifers	2250 dairy cows 850 heifers
Average milk production	10 200 kg/cow/year	9800 kg/cow/year	11500 kg/cow/year
Health status at the beginning of the program	TB, brucellosis and leucosis free, IBR vaccinated	TB, brucellosis and leucosis free, IBR vaccinated	TB, brucellosis and leucosis free, IBR vaccinated
Breed	Holstein Friesian	Holstein Friesian	Holstein Friesian
BVDV vaccination at the beginning of the program	No	No	No
Culling rate 2021	42%	48%	40%
Duration of blood sample collection	04/2021-12/2022	05/2021-12/2022	06/2020-12/2022

Follow-up monitoring investigations were performed by serology and qPCR, as indicated in Fig. 1-3.

The partial nucleotide sequences of the genome region coding for the Npro protein was determined for the detected viruses and used for further comparison as described earlier ([KISS et al., 2022](#)).

The herds involved were never vaccinated before the investigation period and no animals entered the farms in that period.

Results

Most of the blood samples were ELISA negative. Milk samples were received from only

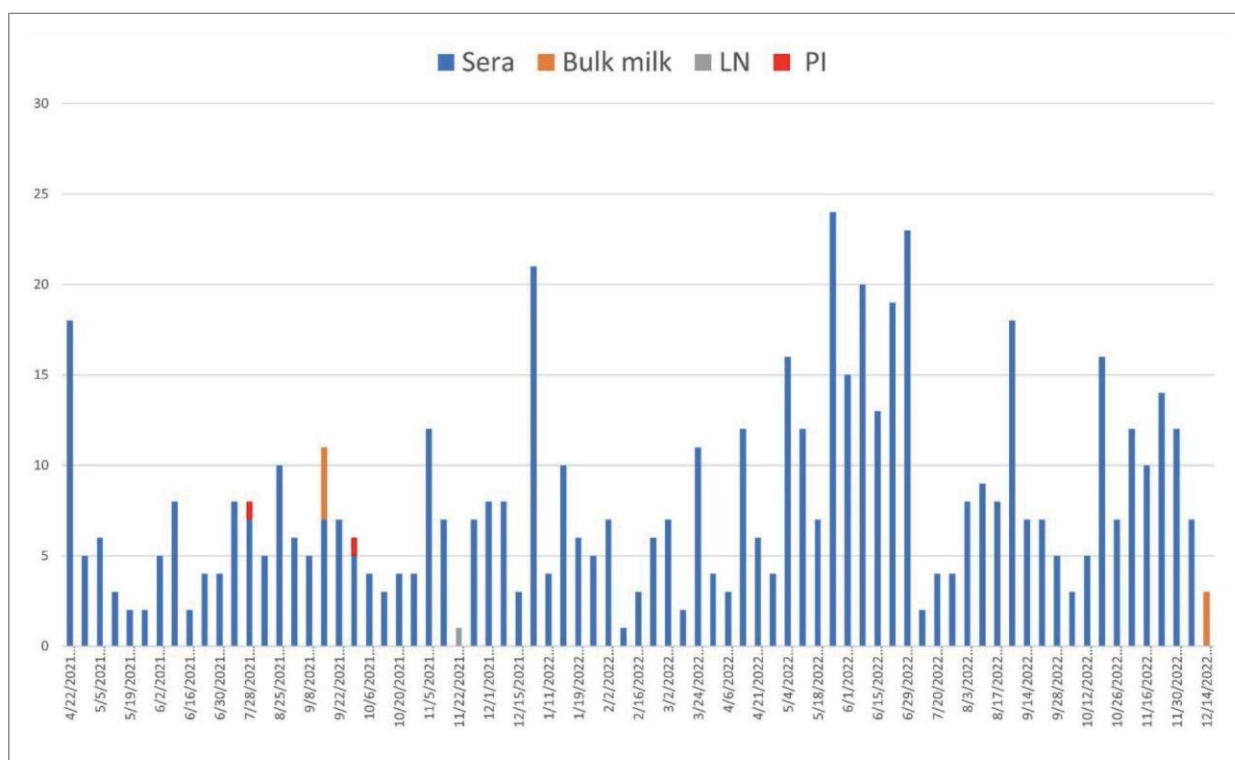


Fig. 1. Sequential presentation of the type/number of tested samples, PI animals identified on farm A

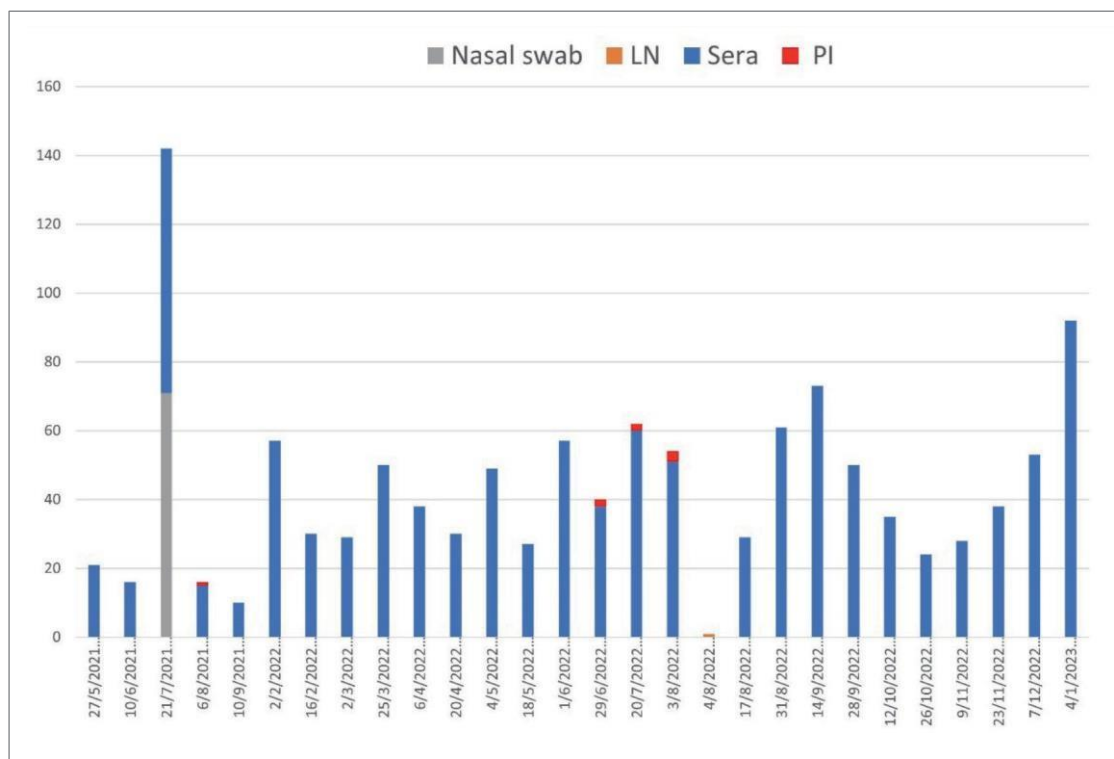


Fig. 2. Sequential presentation of the type/number of tested samples, PI animals identified on farm B

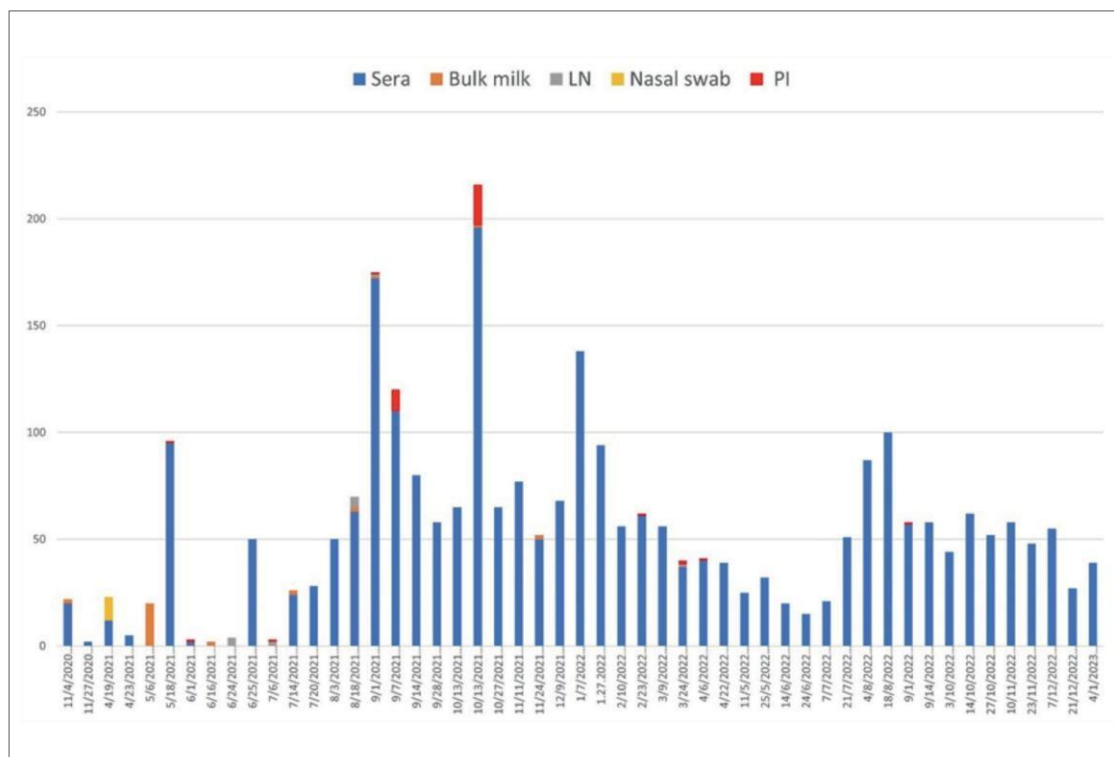


Fig. 3. Sequential presentation of the type/number of tested samples, PI animals identified on farm C

two farms, and sporadically, which resulted in PCR positivity in a few cases.

Nine PCR positive animals were detected out of 730 (1.2%) serums tested on Farm A, 16 PCR positive animals were found out of 1034 (1.5%) animals on Farm B, and 36 PCR positive animals out of 2664 (1.3%) on Farm C. Two out of nine (2.2%) calves were born PI from PI dams on Farm A, three out of 36 (8.3%) from PI dams on farm C, and no calves were born from PI dams on Farm B. There were 77.8%; 100% and 66.7% Trojan births, respectively.

The BVD-PI prevalence rates at animal level were 0.9%; 1%, and 1.1% on Farm A, Farm B, and Farm C, respectively. PI suspicion was confirmed by repeated testing over a three week interval.

There was a lymph node submission from Farm A in November 2021, which was qPCR positive for Pestivirus A. The virus was isolated and characterized as the ncp biotype. Farm B submitted 71 nasal swab samples in July 2021, all of which yielded rather high Ct values (>33), preventing isolation and further sequencing. The same farm submitted a lymph node sample in August 2022, which was negative for Pestivirus A. Farm C, the largest of the three, submitted lymph node samples on several occasions over time (Fig. 3), which all proved Pestivirus A positive by qPCR. Out of the 11 nasal swabs submitted in April 2021, five were Pestivirus A positive.

The following Pestivirus A subtypes were detected on the three farms: 1f and 1d on Farm A; 1b on Farm B; and 1f on Farm C. Interestingly, the 1f virus was detected on Farm A only once, while consecutive samplings confirmed the presence of the same subtype on the respective farms over time. The comparison of the partial Npro coding sequence indicated 100% nucleotide identity consistently (Farm A); 99.21-100% of both nucleotide and amino acid homology (equivalent with 3 and 1 nucleotide and amino acid substitutions) on Farm B; 98.19-100% nucleotide homology (5-0 substitutions), and 100% amino acid homology on Farm C.

Discussion

The low rate of ELISA seropositivity at the beginning of the process was in accordance with

the lack of vaccination, but also with the presence of the virus in the herds. The PCR positive milk samples on Farms B and C were indicative of PI animals in the respective herds, which was later confirmed by individual testing.

A large scale analysis reported that between 1980 and 2016 the worldwide PI prevalence of BVDV decreased from 1.85% to 0.36% at animal level, and declined from 42.36% to 18.88% at herd level ([SCHARNBOCK et al., 2018](#)). The present study demonstrated 1.2% to 2.2% BVDV PI prevalence in three Hungarian industrial dairies, which correlates with the respective European data. The latest study found high (7.2%) within-herd virus prevalence in Hungary ([SZABARA et al., 2016](#)). Both this, and our data indicate that the prevalence of BVDV at animal level has slightly decreased over the last decade in Hungary. There are 32 industrial dairy herds with more than 1000 dairy cows in Hungary, and the concentration of the Hungarian dairy population is one of the highest in the whole of Europe ([DOBOS et al., 2020](#)). As a result, the Scandinavian control strategy would not suit the Hungarian situation. Ensuring the biosecurity level of the dairy, removal of PI animals, and monitoring of herd status, in combination with systematic vaccination, provide a sound basis for controlling the disease. As there are many susceptible cattle on large industrial farms, there is a higher probability of Trojan births, which are the source of BVDV. The potential risk of BVDV infection (the defined window of susceptibility to BVDV is days 30-120 of gestation) is high, mostly on large industrial dairy farms, where there are many cows within this gestation period at any one time. In accordance with the literature data, our results also demonstrated that the major source of PI calves are non-PI dams, the so-called Trojan cows, themselves being virus negative, while harboring PI offspring ([ALBRECHT et al., 2021](#)).

Several studies have highlighted why BVD is widespread despite intensive vaccination ([MOENNIG et al., 2005](#); [WERNIKE et al., 2017](#); [SOZZI et al., 2020](#)) Genetic variation of viral strains and antigenic variance may be responsible for the disappointing results of vaccination ([NEILL et al., 2011](#); [ZIMMER et al., 2002b](#)). For this reason,

we performed the antigenic characterization of representative virus isolates from each farm in cross virus-neutralization tests, using the sera of cows previously negative for BVDV, as well as antibody ELISA, and those who had been immunized with Mucosiffa several times, and we showed cross reactivity between the vaccine induced humoral immune response and the different field strains ([KISS et al., 2022](#)). This information encouraged the involved farms to embark on the control of BVDV infection using vaccination as part of the relevant toolkit, to provide the necessary immune status for the dams, to prevent the emergence of PI animals. This concept was validated by the follow-up monitoring tests, which confirmed the clearance of virus resources (i.e. PI animals) and the progressing seroconversion of the animals. Use of a killed vaccine subtype 1a vaccine proved dissatisfactory for the control of BVDV infection in Polish herds, regardless whether PI animals were removed or not ([ANTOS et al., 2021](#)).

The investigation also provided useful data on the herd specific strain enigma, addressed previously by others and resulting in contradictory findings ([HAMERS et al., 1998a](#); [1998b](#)). On all three involved farms we found that the prevalent virus strains retained their Npro amino acid sequences, although there were several silent nucleotide substitutions on Farms B and C over time. This was a fairly limited scale sequence analysis and should be considered rather as an indication than as sound evidence regarding the stability of the prevailing strains.

Conclusions

In conclusion, we demonstrated that antigenic cross reactivity between a vaccine and differing field strains could readily be used on infected farms, and the identification and systematic removal of PI animals, supported by vaccination, and monitoring the progress of the program lead to the eradication of BVDV infection in less than two years.

Ethics statement

From an ethical perspective, the material collected and used as part of this study was outside

the scope of Directive 2010/63. All methods were carried out in accordance with the relevant guidelines and regulations, and the manuscript adheres to ARRIVE guidelines.

Declaration of competing interest

The authors declare no potential conflicts of interest with respect to the research, authorship, or publication of this article.

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Received: 8 April 2024

Accepted: 7 June 2024

Online publication: 31 December 2024

DOBOS, A., K. SZIGETI, L. CUBAS-GAONA, H. SASVÁRI, M. SAMARDŽIJA, M. KOVACIC, I. KISS:
Učinkovita kontrola virusnog proljeva goveda na velikim industrijskim farmama mliječnih krava zaraženih
različitim podtipovima pestivirusa sojeva A. Vet. arhiv 95, 149-156, 2025.

SAŽETAK

Virusni proljev goveda (BVD) gospodarski je važna bolest u stočarskoj proizvodnji, koja je raširena diljem svijeta, a uzrokovana je pestivirusima tipa A i B te, rjeđe, pestivirusom tipa D. Infekcija virusom ima osebujnu epidemiologiju koja uključuje imunotolerantnu, perzistentno inficiranu (PI) telad, koja izlučuje virus u velikoj količini. Stoga je otkrivanje i iskorjenjivanje virusa temelj eradikacijskih programa, a navedeno se provodi uz potporu dobrog imunosnog statusa gravidnih ženki kako bi se spriječila pojava perzistentne infekcije u teladi. Prikazali smo učinkovitu kontrolu prevladavajućeg pestivirusa A, podtipova 1b, 1d i 1f, na različitim industrijskim farmama mliječnih krava u Mađarskoj. Pri tome slijedila su se načela iskorjenjivanja virusa koji uzrokuje virusni proljev goveda (BVDV), kao što je probir stada na prisutnost i uklanjanje perzistentno inficiranih životinja, odgovarajuća vakcinacija i praćenje BVDV-a u stadu laboratorijskim testovima. Analizirani dijelovi kodirajućih sekvencija Npro uputili su na prisutnost sojeva specifičnih za stada na svakoj farmi. Rezultati su potvrdili da je cijepljenje moćan alat u iskorjenjivanju BVDV-a, čak i ako je živi cijepni soj heterologan u odnosu na cirkulirajuće sojeve na farmama s inficiranim životinjama.

Ključne riječi: mliječna goveda; BVD; kontrola; sekvencija Npro
