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SEVERE ADVERSE IMPACT OF BOVINE VIRAL DIARRHEA ON CATTLE PRODUCTION: A COMPREHENSIVE APPROACH TO CONTROL

ŠTETNI UTICAJ BOVINE VIRUSNE DIJAREJE NA PROIZVODNE KARAKTERISTIKE GOVEDA: SVEOBUHVATAN PRISTUP KONTROLI

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ABSTRACT

Bovine viral diarrhea (BVD) is caused by a pestivirus known as BVDV and is one of the most important infectious diseases of cattle, with a huge economic impact worldwide. The most important source of infection are persistently infected (PI) and diseased cattle. In addition to cattle infection, BVDV infection has been diagnosed in sheep, goats, pigs and wild ruminants (roe deer, deer, bison), as reservoirs of the virus and sources of infection in cattle herds. The consequences of BVDV infections are abortions in pregnant animals, poor female conception, mummification and congenital malformations of fetuses, respiratory problems, transplacental infections and fetal death, neonatal and postnatal mortality, mucosal diseases, slowed growth and poorer performance of surviving animals. Hemorrhagic syndrome (with thrombocytopenia and hemorrhage) is caused exclusively by non-cytopathogenic (NCP) BVDV genotype 2, i.e. virulent strains. The basis of the control program is the prevention of intrauterine infection by identifying and removing PI animals from the cattle herd. The high prevalence of BVDV in cattle worldwide and in Serbia is a danger and causes extremely high economic losses, preventing international trade in breeding and fattening cattle with EU countries, primarily due to uneven approaches or lack of control programs. There are suggestions that the control of the disease in Serbia could be based on a voluntary BVDV eradication program at the herd level, based on four phases, which includes frequent diagnostic tests, removal of PI animals from the herd and introduction of strict biosecurity measures. Certification and register of herds with BVDV free status could be done through the Veterinary Administration.

Key words: Cattle, Bovine viral diarrhoea, Pestivirus, BVDV, Prevalence, Disease control, Economic impact

SAŽETAK

Bovina virusna dijareja (BVD) je uzrokovana pestivirusom poznatim kao BVDV i jedna je od najvažnijih zaraznih bolesti goveda, sa ogromnim ekonomskim uticajem širom sveta. Najvažniji izvor infekcije su perzistentno zaražena (PI) i bolesna goveda. Pored infekcije goveda, BVDV infekcija je dijagnostikovana kod ovaca, koza, svinja i divljih preživara (srne, jeleni, bizoni), kao rezervoari virusa i izvori zaraze u govedima. Posledice BVDV infekcija su abortusi kod gravidnih životinja, loše začeće ženki, mumifikacija i urođene malformacije fetusa, respiratorni problemi, transplacentalne infekcije i fetalna smrt, neonatalni i postnatalni mortalitet, bolesti sluzokože, usporen rast i lošiji rad preživelih životinja. Hemoragijski sindrom (sa trombocitopenijom i krvarenjem) izaziva isključivo necitopatogeni (NCP) BVDV genotip 2, odnosno virulentni sojevi. Osnova programa kontrole je prevencija intrauterine infekcije identifikacijom i uklanjanjem PI životinja iz stada goveda. Velika rasprostranjenost BVDV kod goveda širom sveta i u Srbiji predstavlja opasnost i izaziva izuzetno velike ekonomske gubitke, sprečavajući međunarodnu trgovinu priplodnim i tovnim govedima sa zemljama EU, pre svega zbog neujednačenih pristupa ili nedostatka

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programa kontrole. Postoje sugestije da bi se kontrola bolesti u Srbiji mogla zasnivati na dobrovoljnom programu iskorenjivanja BVDV na nivou stada, zasnovanom na četiri faze, koji podrazumeva česta dijagnostička ispitivanja, uklanjanje PI životinja iz stada i uvođenje strogih mera biobezbednosti. Certifikacija i registar stada sa statusom BVDV slobodnog može se obaviti preko Uprave za veterinu.

Ključne reči: goveda, bovine viral diarrhoea, Pestivirus, BVDV, Prevalencija, Disease kontrola bolesti, ekonomski značaj.

INTRODUCTION

Significant economic losses as a consequence of BVDV infections in cattle have been proven and calculated worldwide (1-3). BVDV are a diverse group of positive-sense single-stranded RNA viruses belonging to the genus Pestivirus within the family Flaviviridae (4). BVDV is classified into 2 types or genotypes: BVDV-1 and BVDV-2, and these two genotypes are divided into several subgenotypes BVDV-1a-1k and BVDV-2a-2c2nd (5-8). Recently, phylogenetic analysis identified bovine pestiviruses and classified them into subgenotypes: at least 21 BVDV-1 (1a–1u), four BVDV-2 and four HoBi-like subgroups (9,10). The latest version of the Flaviviridae taxonomy was ratified in 2023, in which the species names were changed in a conciliatory manner: Pestivirus bovis (from Pestivirus A), Pestivirus tauri (from Pestivirus B) and Pestivirus braziliense (from Pestivirus H) (11).

In addition to the division of genotypes, there are also biotypes based on the presence or absence of a visible cytopathogenic (CPE) effect in infected cell cultures: cytopathogenic (CP) or non-cytopathogenic (NCP) BVDV (12). BVDV is present in cattle on all continents and subcontinents, except for small isolated, exotic areas such as Madagascar. The frequency of infection varies from country to country and from herd to herd, and causes high morbidity and mortality resulting in high losses in the cattle industry (13). Cattle infection caused by a pestivirus known as BVDV, or mucosal disease (MD), is characterized by the following clinical symptoms and changes: erosive inflammation of the mucosa of the digestive tract, diarrhea, mucopurulent nasal discharge, fever, and cachexia. Younger animals from 3 to 8 months are especially susceptible. In addition to acute infection, the infection can also be in a chronic form, and often occurs unintentionally, without clinical symptoms and is persistent in nature, accompanied by immunosuppression and immune tolerance. Abortions and poor conception of females, mummification and congenital malformations of fetuses seriously compromise the reproductive performance of breeding cattle in herds (14).

BVDV infections cause respiratory and reproductive problems: transplacental infection and fetal death, neonatal and postnatal mortality, mucosal disease, stunted growth and poor performance in surviving animals (15,16). BVD is most often manifested clinically after 5 to 7 days of incubation and in an acute form, when morbidity is high and mortality in young individuals is up to 90%, while it is extremely low in the elderly. It most often occurs as an infection in a subclinical course. The most frequently affected animals are 6 to 24 months old. MD is a sporadic form of BVD infection in cattle with clear clinical signs and almost always fatal outcome, acute or chronic course. Morbidity is low and mortality extremely high. The disease usually occurs in cattle 6-24 months of age, and disease onset is associated with immune tolerance in cattle persistently infected (PI) with BVDV.

Major economic losses as a result of BVDV infection include the occurrence of PI calves, which can develop fatal "mucosal disease" (17-19). Placental infection with an NCP virus strain in the first trimester of gestation can induce persistently viremic calves (19), while fetal infection later in gestation often causes abortion, delayed development, or healthy virus-free and seropositive offspring (20,18). PI lifelong carriers of the virus play a key role in the epidemiology of BVDV.

Hemorrhagic syndrome (with the occurrence of thrombocytopenia and hemorrhage) is caused exclusively by NCP BVDV genotype 2, high virulent strains. They affect all age categories of cattle. Cho et al., (21) describe the occurrence of severe hemorrhagic diarrhea in two one-year-old Korean cows in the same herd. The first infected cattle had severe dehydration and died after 3 days. Another beef had anorexia, depression and severe diarrhea with mucus and blood and was necropsied. BVDV2a was detected in the tissues of his digestive tract. Extensive lesions (erosions, ulcerations and extensive bleeding) were observed on the mucous

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membrane of the digestive tract. Immunohistochemically, an extremely positive staining for BVDV2a antigen in the large intestine was diagnosed, which all indicated a hemorrhagic disease caused by BVDV2a in an autochthonous Korean animal. BVDV is one of the most enigmatic and problematic viral agents that lead to BRDC in coinfection. BVDV causes infections in domestic and wild ruminants worldwide (4). In addition to cattle infection, BVDV also infects sheep, goats, pigs and wild ruminants (roe deer, deer, bison), which can be reservoirs of the virus in nature, and thus the source of cattle infection (22,23). Cross-infection between cattle and sheep infected with BVDV and BDV has been demonstrated (24-27). Syndrome X, described in sheep in France, has a multifactorial etiology, and BDV and BVDV antibodies were detected by serological methods. In some cases, other pestiviruses were also isolated. Hyena disease of cattle was first reported in France and recognized in many other countries and was described as a skeletal disorder, and BVDV was identified as the cause of this disease (28,29). To date, little progress has been made in understanding the potential transmission of BVDV from cattle or other ruminants to humans.

Two clinical syndromes were previously described in which pestivirus infections are associated with microencephaly in children and diarrhea of noogenic etiology. Low titers of neutralizing antibodies (Ab) to ruminant pestiviruses were detected in dams that gave birth to micro encephalic calves (30). Feces of children suffering from diarrhea of unknown etiology were found to contain BVDV antigen (17).

There is an urgent need to create fast and accurate diagnostic methods for the detection and control of cattle diseases, and today existing methods are significantly improved or new methods are designed. One of the latest and very interesting methods is recombinase polymerase amplification (RPA). This is a nucleic acid level technique different from PCR, which predominantly involves 3 enzymes, namely, binding single-stranded nucleic acid recombinase (T32 UvsX), single-stranded DNA-binding protein (SSB), and strand-replacing DNA polymerase (Bsu polymerase) (31). An interesting development is observed in the study of Jiang et al. (32), an RPA assay was established for the rapid, sensitive and simultaneous detection of BVDV and BoHV-1 without the need for expensive laboratory equipment, which to the best of the authors' knowledge is the first report on the simultaneous detection of BVDV and BoHV-1 using RPA. The advantages over other known detection techniques are time-saving (completed in less than 30 minutes), does not require thermal cycling and is suitable for non-instrumented platforms for nucleic acid amplification. RPA represents a rapid and efficient method for the simultaneous detection of BVDV and BoHV-1, especially for laboratories with limited resources.

PREVALENCE OF BVD INFECTION AMONG CATTLE WORLDWIDE WITH SPECIAL REFERENCE TO THE ACTUALLY STATUS IN SERBIA

Pioneering research on the representation of BVDV in Serbia was carried out by Belić et al. (97), by examining 224 samples of bovine serum from 6 farms located in Banat and the surroundings of Belgrade and Valjevo for the presence of antibodies to neutralize the BVD virus (VN). A positive titer was determined in 74% of samples (1:9 to 1:2187). The percentage of seropositive animals on certain farms was in the range of 38.8% (91%). The high difference in seroprevalence in different farms was related to the housing system and the age of the animals.

Kurćubić (33) conducted research on dairy farms where the percentage of BVDV seropositive animals varied between 30.55 and 52.24%, depending on age category and herd management practices. On another farm with fattening cattle (aged 6 to 7 months), 55.81% of seropositive sera were detected. The first extensive research that detected antibodies against BVDV in the sera of cattle in the Južno-Bački and Srem districts with the VN test was conducted in 1999-2000 (34).

Serum samples were taken from cattle older than 6 months. VN antibodies against the C24V strain of BVDV were tested in 2546 sera and 46.50% of the samples were positive. The presence of VN antibodies against the NADL strain was examined in 2657 sera, with 50.96% of positive samples. The presence of antibodies against AD-8 strain in 2657 sera was positive in 50.77% of samples.

Petrović et al. (35) detected ncp BVDV isolates 0016 and 0017 and cp isolate Belgrade using indirect immunostaining technique. The OIE reference laboratory (Agency for Veterinary Laboratories, Weybridge, Great Britain - letter dated December 9, 2002) confirmed the official

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registration of BVDV infection in cattle in Serbia and Montenegro. Isolates 0016 and Belgrade were genetic subtype BVDV 1f. Isolate 0017 belongs to subtype 1b, which was proven by direct sequencing of PCR products and phylogenetic analysis. Serological examinations of 12,083 blood samples of cattle older than 6 months in Serbia revealed 4,647 (38.46%) positively tested animals (36). Kurćubić et al. (37) were the first to attempt to identify PI cattle with BVDV among dairy cattle that were seronegative in both paired sera assays (day 1 and 28), using serum BVDV antigen (Ag) ELISA testing, but in both assays BVDV Ag not confirmed.

Kurćubić et al. (38) tried to identify the presence of PI in cattle of different ages from herds with different production purposes (fattening/milking). BVDV antigen was not detected even in the tested dairy and fattening cattle. The explanation for the impossibility of detecting PI cattle lies in the fact that the untested serums of all animals from farms whose age permitted this test, as well as the known fact that PI cattle are represented in herds in a very small percentage (0.75-2%).

Kurćubić et al. (39) examined blood sera collected from sheep of different age categories reared in different systems for the presence of BVDV and border disease virus (BDV) infections by VNT. It was an attempt to clarify the possibility of transmission to livestock and vice versa. Samples obtained from sheep are grown on five mini-farms where there was no cohabitation of small and large ruminants in the same facilities and 5 sheep farms where cattle are also raised. Blood samples were taken from 5 young sheep (up to 12 months) and 5 from older sheep per farm. Serological testing did not detect the presence of anti-BVDV specific Ab for both BVDV genotypes in any of the 100 blood serum samples tested, such as anti BDV Ab to the Moredun strain of BDV.

As part of their doctoral dissertation, the immunogenicity of two experimentally prepared inactivated vaccines (mono and polyvalent) containing BVDV reference and field strains was evaluated by Kurćubić et al. (40). Tested fattening calves were divided into 3 experimental groups: 10 calves vaccinated twice (1st and 28th day) subcutaneously (s.c.) with 2 mL of inactivated polyvalent vaccine per animal (I group); 10 calves vaccinated in the same way with inactivated monovalent vaccine (Group II) and 9 unvaccinated calves constituted the control group (Group C). Blood sera were collected from immunized animals by a standard procedure: 0, 14, 28, 42 and 56 days after immunization, and tested by VN test. The immune response developed faster and the geometric mean titer (GMT) values for BVDV specific neutralizing Ab were significantly higher in samples from calves from Group II.

Debeljak et al., (41) in one study confirmed with a comprehensive clinical and pathohistological examination and laboratory molecular analyzes the occurrence of mucous membrane disease in one cattle in the field. The animal is clinically suspected of having mucosal disease during routine epidemiological and clinical observation in the field. Isolation of the virus in the MDBK cell line showed a cytopathogenic (CP) effect after 48 hours from samples of transformed parts of the tongue, gums and dental pad. RT-PCR revealed that the isolated virus belonged to BVDV1a genotype and CP biotype. This was confirmed by comparing the 5' UTR sequence with the sequence of viral representatives of some genotypes of BVDV and other pestiviruses (from NCBI GeneBank), as well as the results of virus isolation in a cell line. The stated facts and realistic predictions of the potential and possibility of spreading BVDV in Serbia indicate the undoubted need to introduce a program to control the infection of cattle with BVDV.

İnce and Sait (42) conducted research over a period of two years (from March 2017 to April 2019) to determine the prevalence of BVDV and detected the virus in cattle in dairy farms in the Central Anatolia region (Türkiye), using molecular methods. In this cross-sectional study, a total of 393 blood serum samples from 24 farms were collected by random sampling. The presence of antibodies was tested by a virus neutralization assay using NADL, the reference strain of BVDV. The samples were checked for the presence of BVDV-specific antibodies (Ab) and titer values with the serum neutralization test. Serum samples were tested for the presence of BVDV-specific antigens and specific RNA with a commercial ELISA kit and RT-PCR method, respectively. Seropositivity at the level of animals and herds was at the level of 55.72% (219/393) and 79.16% (19/24), respectively. Seropositivity was statistically significantly different between age groups (χ^2:11.81; p=0.002): prevalence of 45.13%, 60.53% and 73.07% of seropositive in the age groups of 6 months-2 years, 2-5 years and above 5 years. All tested samples for detection of persistently infected animals were negative for antigen and BVDV-specific RNA. The presence of

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BVDV infection in dairy cattle enterprises in the province of Konya has been proven, so it is recommended that it is necessary for the country's economy to prevent the spread of the infection and implement voluntary eradication programs.

The study of Werid et al. (43) focused to investigate the prevalence and risk factors associated with BVDV infections in cattle populations through a systematic review and meta-analysis. The health condition of the examined cattle significantly influenced the prevalence of BVDV. Cattle with bovine respiratory disease complex (BRDC) showed higher levels of antibodies (prevalence of 0.67) and antigens (prevalence of 0.23) compared to cattle with reproductive problems (prevalence of 0.13) or diarrhea (prevalence of 0.01). Cattle age had an effect on prevalence (higher rates in adult cattle compared to calves). Breeding and reproductive risk factors are associated with increased prevalence. Coinfections with pathogens such as bovine herpesvirus-1 or *Neospora caninum* are positively correlated with a higher prevalence of BVDV. Poor management practices such as mixing, introduction of new cattle and direct contact with neighboring farms affect the spread. Larger herds and the presence of PI cattle are associated with higher prevalence. The study indicated the importance of detection methods and risk factors in epidemiological BVDV studies.

BVDV-1a strain was detected as the main subtype in cattle imported from Australia to West Java, Indonesia. BVDV infection includes 12 characteristic symptoms synergized with a positive pattern in PCA, all of which may facilitate the development of preventive/control measures to limit the circulation of BVDV in Indonesia (44).

Birhanu et al. (45) conducted a cross-sectional study of 337 blood serum samples originating from 17 randomly selected farms (out of 133 registered dairy farms in Ethiopia), with the aim of detecting seropositive cattle and BVDV antigen in herds without a history of BVDV vaccination. Antibodies against BVDV were detected by indirect enzyme-linked immunosorbent assay (I-ELISA). In contrast, an antigen capture ELISA has been used to detect BVDV antigen in seronegative animals. The total seroprevalence at the animal level was 15.4%, and 64.7% of herds had at least one seropositive animal. Out of 285 seronegative animals, one animal (0.4%) was positive for BVDV antigen. The same animal was found to be positive by double checking after 21 days. In this study, cows with a history of abortion (OR = 6.3; 95% CI: 1.61 -13.1), a history of repeated breeding (OR = 7; 95% CI: 2.5 - 14.3), animals intensive care (OR = 4.6; 95% CI: 1.6 - 13.0) and multiparous cows (OR = 3.6; 95% CI: 1.5 - 8.9) had a higher proportion of sero-reactors in its comparison category ($p<0.05$). Cows that gave birth to calves with congenital defects (OR = 15.2; 95% CI: 3.2 - 73.6), adult head age groups (OR = 2.9; 95% CI: 1.0-7.9) and cows raised by both artificial and natural mating (OR = 4.6; 95% CI: 1.7 - 12.6) were statistically associated with BVDV seropositivity ($p<0.05$). Therefore, given the proven presence of PI individuals in stadiums in Ethiopia, an urgent control intervention involving screening, culling of PI animals and vaccination is warranted.

In analyses of Dunowska et al. (46), the New Zealand 5′ UTR sequences were closely related to each other, although they did not form one well-supported cluster in a phylogenetic tree and BVDV from New Zealand belong predominantly to the BVDV-1a genotype. The majority were most closely related to international BVDV-1a sequences, with the others few clustering with international BVDV-1c sequences.

Of the BVDV genotypes proven to date, the highest prevalence in China cattle is recorded by BVDV1 (47).

Nugroho et al. (48) reported that the BVDV is endemic in 10 Indonesian provinces, with recognized differences in prevalence between regions. The predominant BVDV subgenotypes circulating in cattle herds in Indonesia are BVDV-1a and BVDV-1c. The most likely sources of BVDV transmission in Indonesia are live cattle imported from Australia or contaminated semen from diseased bulls used in artificial insemination. The impact of this pathogen is most manifest on the reproductive performance of cows. Central government needs to facilitate national regulation, surveillance and incentives to implement strategies to control BVDV infections in different sectors within the Indonesian livestock industry.

PROGRAMS FOR THE CONTROL OF BVDV INFECTIONS

The main purpose of implementing control programs is to prevent intrauterine infection by identifying and removing PI animals from cattle herds. Two different types of BVDV infection control are described in the literature, where the first type involves the identification and removal of PI

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animals and all known sources of infection, as well as the frequent implementation of diagnostic control procedures, which should identify and indicate herds absolutely free from BVDV infection. Strict biosecurity measures are applied to prevent PI animals from entering the herd by testing cattle for BVDV. Another type of control involves vaccinations (with different types of vaccines) to ensure an adequate level of immunity in the herd and bull expedient application of milder control measures (49-51). All programs have advantages and disadvantages, but the precise costs and economic impact of each created strategy must be determined, using different models and methods (52-55).

Certain EU countries have implemented control strategies for BVDV, with a long-term aim of eradicating the virus from national herds (56). In the Scandinavian countries, Austria and Switzerland, programs to control BVDV infection in cattle without vaccination are in force. Another type of control program includes vaccination as an optional and additional means of control and is implemented in Germany, Belgium, Ireland and Scotland. A third type of control program to control BVDV infections in cattle is combined programs (57).

Barrett et al. (58) revealed the potential benefits the eradication of BVD will bring to the control of BoHV-1. In Europe, there are two basic types of systematic programs for the control of BVDV infections, those that allow and those that prohibit vaccination. Belgium, Germany, Ireland and Scotland have a similar approach: removal of PI individuals and optional vaccination. The ultimate goal of vaccination is to prevent fetal infection and the emergence of new PI calves. Most countries with systematic BVD control programs without vaccination have reported serious economic damage from the reintroduction of BVDV into cattle populations that have become seronegative and vulnerable after the removal of PIs as "natural vaccinators". Strict biosecurity measures are necessary to prevent re-introduction of the infection into the fields, along with education and continuous motivation of farmers (59).

A BVDV infection control and eradication program was pioneered in the Scandinavian countries in the early 1990s, which included the application of cost-effective diagnostic methods. In general, vaccination was never an option for their decision makers. Considering the fact that vaccination against BVDV was never applied in those countries, it was possible to apply serological methods to identify herds with active BVD infection. The development of ELISA techniques for the detection of BVDV antigens and virus-specific antibodies has enabled cost-effective, low-cost mass screening (60).

Norway was the first country to eradicate this disease in 1993. Combined milk samples from 26,430 samples were examined by indirect ELISA test. Other Scandinavian countries followed suit. The control program was based on: 1) identification of the free herd 2) prevention of infection in this herd and 3) reduction of the number of infected herds. The reduction in the number of infected herds was achieved by identifying and removing PI individuals from the herd, as well as by preventing the possibility of acute infection (61). In Sweden, the control program started in 1993 (62). The Danish program was designed on the same principles and was launched in 1994 (61,63). At the very beginning of the control program, Finland had a huge advantage in a very small number of herds (only a few) positive for the presence of BVDV, and the control program was implemented in the period from 1998 to 2004 (64).

A voluntary BVDV control program called "BVDV-free" has been implemented in the Netherlands since 1997 and surveys in dairy herds were conducted in the interval from August 1, 2007 to August 1, 2013 (65). The program is designed to be implemented at the herd level with a "test and culling" approach, after which BVDV status is monitored by testing young stock for BVDV antibodies or antigen testing of newborn calves. A serious challenge in implementing the program was the lack of legal frameworks or subsidies, so it was hard to motivate farmers to pay all the costs. The only clear benefit for farmers was the eradication of BVDV from their farms. During the above study period, the percentage of dairy farms in the Netherlands with a "BVDV-free" status increased from 13% to 24%, and the prevalence of active BVDV infections in Dutch dairy herds decreased, which may be correlated with the increasing number of participants in the program "BVDV-free".

Yu et al. (66) investigated the economic (gross margins) and production effects (milk yield, somatic cell count and calving interval) in herds certified "BVDV-free" from Dutch dairy herds between 2014 and 2019, in the final stages of the Dutch national program "without BVDV". This study was designed as a case-control study: the case herds in the first

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analysis are those whose BVDV status changed from "BVDV free" to "BVDV free" during the study period. In another analysis, herds started participating in the Dutch "BVDV-free" program during the study period and received a "BVDV-free" certificate. Control herds in both analyzes were "BVDV free" throughout the study period. The results show that there was no significant change in milk yield, somatic cell count, calving interval and gross margin after "BVDV-free" certification. In this study, the effects of "BVDV-free" certification may have been underestimated, given that the Dutch BVDV control program became mandatory during the study period, and some of the case herds may never have experienced any BVDV infection.

The results suggest that in the final phase of the BVDV control program, the program may no longer have a clear benefit for the herd performance of the participating dairy herds. When designing national programs for the eradication of BVDV, it is necessary to include incentives to motivate the owners of such farms to join the program.

Based on the satisfactory results of the control program in the Scandinavian countries, Austria started implementing a very similar program in 2004 (67) and Switzerland started implementing the program in 2008. The strategy differed from that shown for the Scandinavian countries, due to the high prevalence of BVDV (> 80% of seropositive cattle). During the first year of program implementation, all cattle at the national level were virologically tested and proven PI cattle were removed. From 2009-2012, all newborn calves were analyzed for BVDV earwax samples. The applied strategy and specific measures reduced the prevalence of PI from 1.3% to 0.02% (68). Serological monitoring has been carried out since 2013. Dairy herds are monitored with mass milk samples. Animals in fattening herds are monitored by serological blood testing.

Vaccination against BVD is prohibited in the Scandinavian countries, Austria and Switzerland.

The main risk factors for the spread of BVD virus infection are the sale and other movement of animals between herds, live attenuated/modified vaccines (MLV), semen and embryos. Prevention of the import of infected sperm is achieved by regular serological examination of seronegative bulls and/or examination of the sperm of all seropositive bulls (69). Regulations for artificial insemination centers in the EU introduced strict control of bulls against BVD infection (61). Embryos are dangerous if they are not properly washed and the donor is either a PI or an acutely infected animal. Attenuated vaccines are also a constant threat due to frequent contamination with BVD viruses of both genotypes (69). One of the major factors hindering the development of universally effective BVDV vaccines is their genomic diversity (70).

All the mentioned programs implemented in the Scandinavian countries have provided affirmative results. After several years of program implementation, most of these countries were in "BVDV-free" status. Subsequent analyzes revealed that the implementation of the program was cost-effective (71). The control program without the use of vaccination has a weak point in the final phase, after the removal of PI animals, when the spread of BVDV in the herd is reduced and the remaining animals are seronegative. Reintroduction of BVDV into susceptible herds results in maximum damage in terms of acute clinical disease and effects on fertility.

In the Netherlands, a voluntary BVDV control program has been established in 1997 (cattle herds can obtain a BVDV-free status after a full herd screening for BVD virus). Since 2018, the control program became mandatory for dairy herds. The BVDV national herd-level prevalence in dairy herds in the Netherlands declined from 26% (2004) to 8.7% (2016) and the number of BVDV-free and BVDV-unsuspected herds increased. The risk of (re)introduction of BVDV through the purchase of cattle in herds participating in the national Dutch BVDV-free program is limited. However, the (re)introduction of viruses can have a large impact and result in large economic losses for an individual herd. In a country or region that has a successful BVDV control program, the prevalence of BVDV will decrease, leading to an increased proportion of susceptible cattle in the population. In such a situation, the impact of a new outbreak increases, and thus the importance of controlling the risk of purchase. Therefore, to support BVDV eradication in the Netherlands, it remains important to limit the spread of new BVDV infections through cattle introductions. Testing purchased cattle for virus and antibodies was probably helpful in preventing the spread of infection. This conclusion may also apply to other countries where there is a BVDV control program (72).

Very interesting and practical results were reported in their study carried out in Australia by McMorrow et al. (73). The responsibility and costs

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of controlling and preventing BVDV infections are solely borne by the manufacturer. Veterinarians support farmers and impart knowledge on farm-specific BVDV management practices and facilitate regional BVDV control and elimination. Veterinarians were surveyed to determine their knowledge, attitudes and practices (KAP) related to BVDV control in South East Australia. It was discovered that the recommendations of veterinarians are not always in line with the control and biosecurity measures implemented by farmers. Veterinarians were uncertain about the prevalence of BVDV and the percentage of producers implementing BVDV control measures in the regions where they provide support and health care. Veterinarians generally promoted biosecurity and vaccination, and were concerned about the welfare and additional disease risks associated with persistently infected (PI) cattle. Veterinarians have raised concerns about the risk of disease associated with lack of traceability (a previously undocumented practice whereby producers collected blood from PI cattle to administer to cattle that did not test positive for BVDV, termed "vampire vaccination" in this study). A deeper understanding of the burden, impact and economics of BVDV is needed to align veterinarian and producer KAP and improve on-farm management of BVDV. More mutual appreciation of the values of veterinarians and producers is needed before BVDV control can be implemented at a regional or national level.

Strict application of biosecurity measures must be the basis of any control plan to eradicate BVDV. Cattle vaccination as the basis of a control program is interesting because it is a cheap and relatively effective method for preventing infections with the virus that causes BVD. The most important goals of cattle vaccination are protection of the fetus, prevention of clinical diseases and immunosuppression. The most widely used is the modified live vaccine (MLV) against BVD, monovalent or polyvalent - in combination with other viral or bacterial agents (74). Most modern MLVs are created from cp BVDV, because cp viruses are not risky and do not lead to PI fetuses. They induce strong humoral and cellular immunity and provide solid fetal protection. In the EU, the use of MLV in unvaccinated animals during the first 6 months of pregnancy is not recommended. 7-week-old calves can be vaccinated against BVD because after vaccination they develop a T cell-mediated immune response despite circulating maternal antibodies (75).

Safety concerns have prompted the development of inactivated-killed vaccines (KV), which could be administered at any age and period of pregnancy (76). KVs have a disadvantage compared to MLVs because they must be given multiple times to achieve protection. Immunity after KV administration occurs after at least 3 to 4 weeks, while MLVs provide protection within a few days. Fetal protection varies from incomplete to satisfactory (77,76). The immune response to KV has recently been enhanced by the addition of potent adjuvants. Humoral immunity after KV administration is usually strong, and cellular immunity varies from incomplete to strong (78,79). Killed BVDV vaccines (KV) are predominantly present on the EU market. Another disadvantage of KV is the necessary annual revaccination. Vaccine administration has not changed BVD prevalence over time (80), due to unique biology of BVDV infections, and ability to produce PI progeny creates a virus reservoir that is continuously shedding large amounts of infectious virus. Thus, PI cattle have ample opportunity during their lifetime to meet and infect new susceptible animals, even when the general level of immunity against BVD is high. This is in contrast to most other infectious diseases where shedding is temporally limited to a few days or weeks. If PI animals are present in the herd, only a herd immunity of 100% will prevent the emergence of new PI calves, which is impossible to achieve under practical conditions. The consequence of the aforementioned facts is that so far there is no data globally on the successfully implemented control strategy for the control of BVDV bovine infections, which is based exclusively on the implementation of vaccination.

Combined control programs were implemented in Germany between 2004 and 2011, in regions with a dense livestock population (about 200 animals per km^2) and intensive trade, and involve a combined approach of testing and culling PI animals plus optional vaccination. The local epidemiological situation dictates the measures that will be prescribed by the veterinary authorities, i.e. prohibition or recommendation of vaccination.

The implementation of combined programs gave good results: the prevalence of PI cattle decreased from 0.48% in 2011 to 0.02% in 2016 (98). Strategies for the systematic eradication of BVDV infection have been launched in a number of

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European countries, created by the government and the relevant authorities or farmers' associations with the support of the government. They are similar to the above, with certain minor modifications. In 2019, the average farm in Slovenia had 15.8 animals. Of the total cattle population, 29.9% is represented by the Simmental breed, 16.8% by Holstein, 4.4% by brown and 0.9% by the Slovenian autochthonous Cika breed. The rest (48.0%) are either crossbred cattle or those of unknown pedigree or fattening breed of cattle (mainly Limousin, Charolais or Angus). The cattle population consists of cows (34.0%), calves (29.8%), heifers (20.8%) and bulls (15.4%) (81).

In Slovenia, a BVDV-free voluntary control program with provisions for recognition, acquisition and maintenance of the status was adopted for the first time. According to the data from the first 7 years of running the program, only a small part of the herd has an officially recognized and maintained BVDV-free status. The program is paid exclusively by farmers, with discounts and efficient work of the Laboratory of the Veterinary Faculty in Ljubljana; the health condition of several herds was improved by the application of the adopted Rulebook. The number of herds with official BVDV-free status is still very small, which nevertheless benefits farmers who sell and buy animals. Also, the voluntary program provides data on the prevalence of BVDV and the estimated number of BVDV positive herds. Austria and Switzerland have already achieved BVDV-free status at the national level. The next step for Slovenia is to move to a mandatory national program to eradicate BVDV and the diseases it causes. However, during the implementation of the first voluntary BVDV control program, several cattle herds achieved significant improvement and progress in health status after implementing preventive measures or successfully maintained BVDV-free status for several years (82).

In an interval of 7 years (2014-2020), a total of 348 herds were included in BVDV antibody testing. A quarter of the examined herds were positive for the presence of BVDV antibodies. In order for the herd to be in "BVDV-free" status, two consecutive ELISA tests at least 6 months apart in all animals aged 7 to 13 months must have negative results for BVDV antibodies (82).

In the same program, 236 herds were involved in the detection of BVDV in individual herds by the RT-PCR method in real time, in order to identify and eliminate positive animals from the herd. At least one animal positive for BVDV was detected in 31.3% of herds, with a total of 267 (2.9%) persistently infected (PI) animals identified. The costs of testing for an average herd, recognized as BVDV-negative, and maintaining the BVDV-free status within the implemented voluntary program, amounted to €97.64/year. For the average positive herd, the laboratory costs for BVDV elimination were €189.59/year and were borne by the farmers themselves, with limited progress towards eradication at the national level (82).

A longer description of the implementation of the BVDV infection control program in Slovenia can be illustrative of the creation of a similar program in Serbia, which is currently missing on national level.

In the Republic of Serbia, the situation is challenging due to the proven high prevalence of BVDV (especially in fattening cattle), different strains of the virus isolated by modern methods and classified on the phylogenetic tree, clinical cases described. The problems have not been comprehensively solved. Control of BVDV infection in cattle herds is sporadic, there are no systemic solutions. This economic and health problem, which seriously affects the competitiveness of Serbian cattle breeding, was recognized by veterinary experts and the leadership of the Veterinary Administration of the Ministry of Agriculture and Environmental Protection, by launching an initiative to establish a voluntary program for the control of BVD infection on the territory of the Republic of Serbia (83) and the development of the working version of the program for the control, suppression and eradication of BVDV infection in the Republic of Serbia was entrusted to the Scientific Institute of Veterinary Science "Novi Sad" (NIV-NS), within the technological development project no. TR 31084. The program was initially intended to be implemented on a voluntary basis on individual farms and cattle herds.

The BVDV infection control program combines successful methodologies developed and applied in several countries (Scandinavia, Germany, Italy and Slovenia), as well as "modern" diagnostic and laboratory methods that will be included in animal testing (84). If it shows good results, the Program would subsequently be implemented at the national level as a state program for the control of BVDV infection in the Republic of Serbia, with the main goal of herds obtaining the status of "free from BVDV" infection. Herd certification would be verified by the Veterinary Administration, which

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would create an online database of certified herds with all relevant data. The program design includes four phases: Phase 1: Confirmation of BVDV-free status, Phase 2: Gain of BVDV-free status, Phase 3: Maintenance of BVDV-free herd status, and Phase 4: Loss of BVDV-free status. and the procedure for restoring and restoring the herd's BVDV-free status.

The objectives of the program are:

• Reduction of economic losses in cattle herds;

• Obtaining certified reproductive material to facilitate and promote its access to the international market and

• Better market price of certified reproductive material.

In light of the facts presented in this review, we also point to an excellent and invaluable contemporary review based on the vast experience of the authors (85) in which the authors state that volunteer programs are particularly difficult to manage because they require a high degree of awareness, motivation and compliance on the part of interested parties, especially participating farmers. Many new programs are the result of discussions with stakeholders. Instead of adopting a simple and rigorous control program based on the positive experiences of other countries, many compromises are often made for ostensibly economic reasons, which actually jeopardize control efforts from the outset. Typical examples are unclear or non-existent regulations regarding the fate of proven PI cattle, weak or no provisions on movement restrictions and biosecurity, and lack of livestock quarantine rules.

If similar shortcomings exist, long program duration, high costs and poor performance, and very often failure, are inevitable. Successful volunteer programs require a strong commitment from all stakeholders and a desire to make the program successful. A steering group of key stakeholders should facilitate communication with farmers and coordinate education and control measures. During the implementation of all phases and activities of the entire program, efforts must be monitored, while respecting the importance of data management. In certain countries, existing livestock databases have been successfully used to support BVD control.

ESTIMATION OF ECONOMIC LOSSES

Cattle diseases caused by BVD infection are the result of transplacental infections that cause fetal death, congenital malformations, neonatal and postnatal mortality, leukopenia and immunosuppression, respiratory syndrome, reduced milk yield, reduced female conception (permanent mortality, periodontal disease), abortions, mummification, immunotolerance and persistent infection (PI), mucosal disease (MD), poor growth and performance of surviving animals (86-89). In southeastern parts of Norway, BVDV infection has been identified as the cause of 15% of all abortions and stillbirths in dairy cows (99).

Calculation of herd-level losses in so-called classic attacks, where many transient infections go unnoticed, and where herd losses are associated with reproductive disorders and PI animals at the level of $25-160 per cow (100). Losses during attacks when BVDV co-existed with other infections or highly virulent strains causing severe disease and high mortality, as well as transiently infected animals, were calculated to exceed $400 per cow (90, 100). Estimates at the national level (in Great Britain, Norway and Denmark) and subsequent recalculation of calving losses suggest that they were on the order of $10-40 per calving (91, 101). Losses when a virulent strain of BVDV is present in the population are estimated at $57 per calf (13).

In the UK, the annual total economic loss from BVD infection is around £120 million. In Denmark, the damage is estimated at 17 million dollars per year (61). BVDV infections are considered the third most economically important disease in livestock, just behind rinderpest and foot-and-mouth disease. The economic consequences of a national eradication program in Norway are expressed as cost-benefit calculations and show that the program was profitable as early as the second year after initiation, and then had a net present value of $6 million over the first 5 years, 1993-1997 (92). The net present value for the entire eradication period was later estimated at approximately $22 million (93). In Denmark, the costs of a medium-term control program were about $3.5 million—low costs compared to the avoided losses—gains of $20 million, calculated before the program began (13).

FUTURE DIRECTIONS

To study BVDV NSP, it is necessary to investigate which host cell genes should be activated during BVDV transcription, as well as which host cell components are necessary for its translation. Some BVDV NSP genes have a role in transcription, translation and regulation of the virus itself, as well as a role and mechanism in host cell antagonism. For

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the complete prevention of BVDV, it is necessary to strengthen the research of the interaction between BVDV and the host, as well as the interaction between viral structural proteins and non-structural proteins after the entry of BVDV into cells, which is of great importance for the prevention and control of BVD (94).

Workman et al. (95) used CRISPR-mediated homology-directed repair and somatic cell nuclear transfer to generate a live calf with a six amino acid substitution in the BVDV binding domain of bovine CD46. The result was a genetically engineered calf with dramatically reduced susceptibility to infection as measured by reduced clinical signs and a lack of viral infection in white blood cells. The edited calf appears normal and healthy at 20 months of age with no apparent adverse effects of genetic engineering. This precision-bred proof-of-concept animal provides the first evidence that deliberate genomic changes in the CD46 gene can reduce the burden of BVDV-associated disease in cattle and is consistent with our stepwise, *in vitro* and *ex vivo* experiments with cell lines and matched fetal clones.

The consensus panel agrees that continued investment in BVDV awareness and education is important for successful, efficient and effective BVDV control by cattle producers (96).

Identification and prevention risky behaviour within a BVDV control programme and trace routes of BVDV transmission can be performed by a molecular epidemiological approach.

CONCLUSIONS

It has been clearly shown that various BVDV bovine infection control programs and its eradication can be successfully implemented at the national level (Scandinavian countries, Austria, Switzerland, Germany, UK, Australia, New Zealand, Indonesia). Given the described success of the program and visible progress in the control of BVDV infections, in a time interval whose duration varies depending on the initial condition (prevalence of BVDV and percentage of the PI animals). Visible progressions in the control of BVDV infections have been observed. Control programs gave very significant results, and their success was assessed as good usually after a period of implementation of about 5 years. We believe that there are all the conditions to implement the control program as proposed by experts from the Scientific Veterinary Institute „Novi Sad“, which is based on the experiences of European countries and adapted to the specifics of cattle breeding in Serbia. With the support of the Veterinary Administration of Republic of Serbia, the program would grow from a voluntarily phase to a governmental program for the control of BVDV infection.

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