

# Prevalence of BVD infection in ruminants in Serbia

## Rasprostranjenost BVD infekcije kod preživara u Srbiji

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### Abstract

The aim of this article is to provide a historical summary of worldwide Bovine Viral Diarrhoea Virus (BVDV) prevalence data through a number of studies, review the current knowledge and published data on the presence and prevalence of BVDV infection among ruminants in Serbia, and consequently open questions as to the possibilities for the implementation of the control programme in Serbia.

**Keywords:** Bovine Viral Diarrhoea Virus (BVDV), prevalence, ELISA, Reverse transcriptase - Polymerase Chain Reaction (RT-PCR), Virus Neutralization Test (VNT)

### Rezime

Cilj ovog rada je da obezbedi podatke za istorijski rezime o rasprostranjenosti virusa goveđe dijareje (BVDV) širom sveta kroz brojna ispitivanja, razmatra trenutna znanja i objavi podatke o prisustvu i rasprostranjenosti infekcije BVDV među preživarima u Srbiji, a samim tim i postavi pitanja u pogledu mogućnosti za primenu programa kontrole u Srbiji.

**Ključne reči:** virus goveđe dijareje (BVDV), rasprostranjenost (prevalenca), ELISA, reverzna transkripcija – lančana reakcija polimeraze (RT-PCR), virus neutralizacioni test (VNT)

## Introduction

Bovine viral diarrhoea virus (BVDV) is a non-arthropod-borne member of the *Flaviviridae* family in the genus *Pestivirus* that causes infections of domestic and wild ruminants worldwide (Baker, 1995; Lindenbach, et al., 2007). BVDV infection is endemic in most cattle-producing countries throughout the world, causing significant economic losses to the cattle industry (Houe, et al., 1993).

Based on antigenic and genetic properties, two species, previously characterised as two genotypes, of the causative virus can be distinguished, BVDV-1 and BVDV-2. Their prevalence varies across the world; BVDV-2 represents about 50% of the isolates in North America, whereas BVDV-1 dominates in Europe, with more than 90% (Ridpath, 2005; Vilcek, et al., 2005). In addition, BVDV-1 and BVDV-2 genotypes have been further divided into subtypes BVDV-1a, BVDV-1b, BVDV-2a and BVDV-2b in North America (Ridpath, et al., 2000, Flores, et al., 2002). More recently, 12 BVDV-1 subtypes (BVDV-1a - BVDV-1l) and 2 BVDV-2 subtypes (BVDV-2a and BVDV-2b) have been identified (Ridpath, et al., 2010).

Besides cattle, BVDV infection can also occur in sheep, goats, swine and wild ruminants (roe deer, deer, bison, etc.). All of these animals can act as reservoirs for the virus in nature and, hence, as the source of cattle infection (Nettleton, et al., 1980; Cvetnić, 1983). There is also experimental evidence of cross-infections between cattle and sheep infected with BVDV and Border disease virus (BDV). Moreover, this type of infection has also been proved to occur due to natural infection (Osburn, et al., 1973; Dahle, et al., 1985; Nettleton, 1986; Brownlie, 1991b).

In France, syndrome X having a multifactorial aetiology has been described in sheep. Serological methods used to identify infected animals have resulted in the detection of BDV and BVDV antibodies and, in some cases, isolation of Pestiviruses. Hyena disease of cattle was first reported in France and since then has been recognised in many other countries and described as a skeletal disorder. BVDV has been identified as the causative agent of this disease (Russo, et al., 1985; Espinasse, et al., 1986; Nettleton, 1990).

Attention is also drawn to previous data on the possible relationship between ruminant pestiviruses and some human infections. Human infections with Pestiviruses are associated with two clinical syndromes: microencephaly in children and diarrhoea of noogenic aetiology. The faeces of children suffering from diarrhoea of unknown aetiology have been found to contain BVDV antigen (Brownlie and Clark, 1990).

The acute infection of cattle with ncp BVDV mostly results in a transient self-limiting infection. The acute BVDV infection can cause gastroenteritis or respiratory and reproductive disorders in cattle. Frequently, the infection can be clinically inapparent (Baker, 1987, Brownlie, 1991a). Major economic losses due to BVDV infection include reduced fertility, abortions, growth retardation and the generation of persistently viremic calves, which can develop fatal "mucosal disease" (Brownlie, 1990; Bielefeldt, 1995; Moennig and Liess, 1995). Placental infection with ncp virus in the first trimester of gestation can induce persistently viremic calves (Moennig and Liess, 1995), whereas fetal infection later in gestation often causes abortion, retarded development or results in healthy virus-free and seropositive offspring (Done, et al.,

1980; Bielefeldt, 1995). Persistently infected lifelong virus carriers play a key role in BVDV epidemiology.

The most severe damages inflicted by BVDV infections on the cattle industry are the direct result of transplacental infection and include fetal death, congenital malformations, neonatal and postnatal mortality, mucosal diseases, retarded growth and poor performance of surviving animals (Bolin, et al., 1985; Roeder and Harkness, 1986).

The aim of this article is to provide a historical summary of worldwide BVDV prevalence data through a number of studies, review the current knowledge and published data on the presence and prevalence of BVDV infection among ruminants in Serbia, and consequently open questions about the possibilities for the implementation of the control programme in Serbia.

### **BVD control in Europe**

Lindberg, et al. (2006) have reported that a group of scientists involved in BVDV control in EU have suggested that 3 central elements of systematic BVD control approaches can be identified: a) biosecurity aimed at preventing reintroduction of BVD infection in free herds; b) elimination of PI animals from infected herds and c) surveillance to monitor the progress of interventions and to rapidly detect new infections. The primary goal of BVDV infection control programmes is to prevent prenatal (intrauterine) infection (Moening, et al., 2005a; Houe, et al, 2006). Two basic types of BVD control programmes have been classified as systematic and non-systematic (Lindberg and Houe, 2005). The first type established typically on a sectoral, regional or national basis, includes identification and removal of PI animals and any other possible source of infection, and frequent diagnostic control procedures, aiming at establishing herds completely free of BVDV. Strict biosecurity measures are used to prevent entry of PI animals into the herd by testing cattle for BVDV. Progress needs to be monitored and evaluated. The other type of BVDV control is carried out on an individual herd basis, involves the use of vaccination to ensure maintenance of herd immunity, and employment of far less stringent control measures (Harkness, 1987; Ames and Baker, 1990; Brownlie, et al., 1991). The use of vaccines may reduce economic losses caused by clinical disease, but does not appear to result in reduction of the prevalence of BVDV infections (Hjerpe, 1990, O'Rourke, 2002).

The first systematic program focused at eradicating BVDV without the use of vaccines were begun in 1993-1994 in Denmark, Finland, Norway and Sweden (Moening, et al., 2005a). Different preconditions (legal support and initial prevalences of herds with PI animals varying from <1% in Finland to 50% in Denmark) it has taken all countries approximately 10 years to reach their final goals. Swedish experience revealed that if the programmes are implemented in a systematic manner and with basic biosecurity and elimination of virus from infected herds and monitoring of non-infected herds, BVDV eradication is possible and profitable (Hult and Lindberg 2005).

Within Europe, prevalence of BVDV varies enormously. The Scandinavian countries (Norway, Sweden, Finland and Denmark) are free of BVDV, whereas in other countries such as The Netherlands and The United Kingdom sero-prevalence estimates exceed 50% (Lindberg, 2003; Moen, 2005a, 2005b). In addition to those EU member states already officially free of disease, other countries (The Netherlands, Belgium, France, Germany, U.K., Spain and Italy among others) are at

various stages of herd certification/eradication (Dubois, et al., 2010; Franken, 2010; Arnaiz, et al., 2010; Moennig and Grummer, 2010; Cavirani, 2010).

European countries in advanced stages of eradication of BVD are Austria and Switzerland. Systematic control have also been implemented to a varying extent in other parts of Europe, like time-limited regional control programmes in France (Joly, et al., 2005), Germany (Moennig, et al., 2005b), The Netherlands (Moen, et al., 2005), the Rome area, Lecco and Como regions of Italy (Ferrari, et al., 1999; Luzzago, et al., 2004), and United Kingdom. Shetland Islands has been eradicated BVDV (Synge, et al., 1999).

Austria has partly followed Scandinavian model (regional project involving the Lower Austria region), and after 7 years the scheme was extended to the entire country in 2004 (Rossmann, et al., 2005). Although vaccines are available in many countries, all programs except in Germany are based on nonvaccination model. Prior to 2011, BVD eradication in Germany was voluntary, and practiced predominantly in lower Saxony (Greiser-Wilke, et al., 2003). On January 1st 2011, a mandatory BVD control programmes was introduced in all German states. The four subtypes that were found in cattle herds in the Czech Republic belong to BVDV-1, and this results improve the strategy of the BVDV control program in cattle herds and stimulate the introduction of a national BVDV/MD eradication program (Robesova, et al., 2009).

The BVD control programme in Switzerland was originally requested from the Federal Veterinary Office (FVO) in 2004, and planned to start in 2007. This programme was postponed until 2008 to ensure it would run smoothly (Presi and Heim, 2010).

Bosco Cowley and collaborators (2012) reported that Animal Health Ireland (AHI), an industry body charged with the national leadership and coordination of production disease issues in Ireland has implemented a voluntary scheme of elimination for PI calves born in Ireland since January 2012. This model is planned to become mandatory.

Successful integrated BVDV control programs will ultimately improve productivity, performance, health, welfare, and ultimately economic return (Grooms, et al., 2009).

### **Brief history of BVDV prevalence worldwide**

One of the first published reports on BVDV prevalence in Europe was that by Dinter and Bakos (1961) in Sweden on 20.8% BVDV seropositive cattle of different age groups. Serological testing conducted by Bogel (1963) in West Germany revealed variations in the percentage of seropositive cattle from 7.6 to 44.6%, depending on the extent to which the herds tested were closed to new introductions. Serological methods used by Kahrs, et al. (1964) identified seropositivity in 53% of the total number of animals tested in the State of New York in the USA. Schaal, et al. (1971) detected 76.4% of the test animals to be BVDV seropositive in some regions in West Germany. The percentage of seropositivity was lower in young animals (39.5% of animals aged 12-18 months to 91.5% of adult animals). In Austria, Sibalin and Burki (1972) performed serological testing and identified 64% seropositive animals aged over two years, over 51.3% animals aged 1-2 years and 41% animals 6-12 months of age. Using the virus neutralisation (VN) test, Phillip (1973) detected that 61% of over 4000 dairy cows in Great Britain were seropositive.

Over 1976-1984, Cancellotti and Carlotto (1985) used the VN method to check about 10000 serum samples as part of his epidemiological surveillance activities. The

results varied due to different age, origin and intended use of the animals. The percentage of seropositive animals went as high as 100% in adult animals. Diagnosis based on antigen isolation and/or detection in the organs of dead animals showed that BVDV was the causative agent of the disease in more than 30% outbreaks of bovine diarrhoea in the Venetia region (covering 1.5 million hectares) during 1976-1984.

In Germany, during 1983-1985, Peters and co-workers (1985) collected blood samples from pregnant cows intended for export from a large number of herds in an attempt to isolate BVDV on cell culture. Out of the 2317 non-suspicious animals, less than 1% of persistently viremic animals were detected. Frey, et al. (1985) performed serological testing of 221 serum samples collected from cattle aged 6-12 months for the presence of BVDV specific antibodies and determined their presence in 86 animals (62%). Gunn (1985) used the VN method to test 1141 bovine serum samples and detected 78% BVDV seropositive samples (antibody titres ranged from 1:16-1:256). The testing conducted by Moennig and Liess (1989) resulted in identification of about 80% BVDV seropositive animals in Germany and 1-2% persistently infected (PI) animals of the "healthy" population.

In Croatia, a study was conducted to determine the prevalence of IBRV and BVDV antibodies in sera obtained from dairy cows on four different farms (Biuk-Rudan, et al., 1998). Antibodies to both viruses were found in 80.8% of cows with reproductive disorders but in only 46.8% of cows without reproductive disorders. The difference was statistically significant ( $p < 0.01$ ).

Thirty-nine Greek dairy herds comprising 6333 cattle in total, were enrolled in a voluntary bovine viral diarrhoea virus (BVDV) eradication programme based on the identification and removal of persistently infected (PI) animals (Billinis, et al., 2005). Antigen positive and PI animals were detected in all herds. The respective mean prevalence, adjusted for test accuracy and the herd-clustering effect, was 14% (95% CI: 11-18%) and 1.3% (0.8-1.8%), respectively.

In South Korea blood samples were collected from 1328 dairy cows of different parities in 46 herds in two regions and tested for BVDV by reverse transcriptase - PCR (RT-PCR) for the detection of viral sequences in whole blood and by a commercial ELISA for the detection of BVDV-specific antibodies. None of the animals was positive by RT-PCR but 770 (58%) were tested seropositive (Lee, et al., 2008).

The prevalence of BVDV PI cattle in beef breeding herds was determined in 30 herds comprising 4530 calves in the South Central United States. Samples were also collected from the dams of PI calves. Twenty five PI calves (0.55%) from 5 of the 30 herds (16.7%) have been detected. The virus subtype of all the PI isolates was BVDV1b. Histories of the ranches indicated that 23 out of 30 had herd additions of untested breeding females. Twenty four of the 30 herds had adult cowherd vaccinations against BVDV, primarily using killed BVDV vaccines at pregnancy examination (Fulton, et al., 2009). Ridpath, et al. (2010) tried to determine the prevalence of BVDV genotypes and subtypes in the United States and Australia as well as detectable antigenic differences between the prevalent subtypes. The analysis suggested that BVDV-1b and BVDV-1c are the most prevalent subtypes in the United States and Australia, respectively.

A cross-sectional study was conducted to investigate the prevalence of bovine viral diarrhoea (BVD) virus using an indirect ELISA test in industrial dairy cattle herds in a suburb of Shiraz in Iran. Blood samples were collected from 952 dairy cows of

different parities from 43 herds in which the vaccination was never used. Five hundred and seventy three (60.19%) cows were ELISA seropositive. However, the true BVDV seroprevalence was 59.46%. All of the herds were antibody positive against BVDV. The prevalence ranged from 37 to 86% within the herds (Ghane, et al., 2010).

A review of prevalence surveys performed in Europe from the late 1970's and into the 21st century shows that BVDV is basically endemic in all countries where no systematic control has been initiated (Lindberg, et al., 2006). Under such conditions, approximately 50% of all herds have PI animals, and 90% of all cattle become exposed during their lifetime. In endemic areas, a high correlation between BVDV prevalence and cattle density has been shown (Houe, et al., 2003).

### **A brief description of the cattle population in Serbia - Demographic factors**

Lukić and co-workers (2013) reported within your case study that Serbia has approximately 9 million hectares of surface (88509 km<sup>2</sup>), of which 5.1 million ha are agricultural land (1.4 million ha are pastures and meadows). First preliminary statistical results from the almost half of the 7.2 million residents of Serbia are located in rural regions, and approximately one quarter of the population in Serbia is working in the field of agriculture. There are 631122 agricultural holdings in Serbia (99.6% are family agricultural holdings). Negative trend in livestock production particularly in Serbia confirmed through decrease in number of livestock unit per ha of agricultural land in years 2007 and 2012 (0.31 and 0.28). Cattle production is dominant and makes 43% of total livestock production in Serbia in year 2012.

Lukić (2012) reported that the number of heads of farm animals in Serbia has been decreasing in general, especially cattle by 2-3% per year during the last decade, accompanied by reduced production of animal products.

Goss and co-workers (2010) analyzing dairy sector in Republic of Serbia, defined four dairy production systems: small upland farms, small lowland farms, medium farms and large lowland farms. First two production systems dominate in Serbian dairy sector, especially in Central Serbia. Their basic properties are: herds with 1 to 5 cows, mostly Simmental breed, low milk yield, usually more than 5 lactations, cows are tethered in barns and milked by hand or portable machine. Small farms with herd from 1 to 5 cows produce the most of milk in Serbia.

Popović and Knežević (2012) reported that small farms producing individually lower quantity and quality of milk, with less important role for bigger processors. Low milk prices and lack of subsidies pushed small farmers more on informal market. The focus of activities in last decade was to improve size and productivity of commercial dairy family farms (with at least 10 cows in herd). Leading examples are companies Imlek, Mlekara Subotica and Somboled providing loans and consulting service for dairy farmers to obtain additional cows, new barns and milking equipment.

According to Popović (2008) a prevalent portion of farms has no prerequisites to produce quality milk, and established a positive correlation between the size of herd, level of average production per cow and price of milk. The larger farms (with 20 and more cows) have advantage in all parameters.

Perišić and co-workers (2011) showed that in Serbia, according to the Inventory from 2002, 97.61% farms had 1 to 5 cow and they owned 87% heads in a total number of cows in Serbia. Average milk yield in registered Simmental cows in Serbia was 4500 kg, and in Holstein Friesian breed around 8700 kg. It is important to state that in

Serbia there is a far less cows under a controlled milk yield (around 5.5% Simmental breed), compared with situation in Germany that about 19% animals of Simmental breed are under milk yield control, in Slovenia about 15 %, Austria about 15% and Hungary about 11%.

Detailed descriptions of the cattle population in Serbia are summarizing in tables 1 and 2.

Table 1. Main structural indicators of agricultural holdings Republic of Serbia  
Glavni strukturni pokazatelji poljoprivrednih gazdinstava u Republici Srbiji

Livestock fund of holdings	Utilized agricultural area, ha					
	≤ 1	1,01–2	2,01–5	5,01–10	10,01–50	50<
Livestock fund, (000) LU	314742	158924	444148	427519	422736	251821
Heads						
Bovine animals	40133	47429	185018	233342	291142	111038
Bovine, under 1 year old	12561	9263	40322	61595	100870	37836
Bovine, 1 – 2 years old	5585	3783	15524	26016	43753	24122
Bovine, over 2 years old	21987	34383	129172	145731	146519	49080
Male heads	658	798	2939	4226	5067	1646
Heifers	2979	3634	15223	18243	19215	6879
Dairy cows	17479	28988	107331	119935	119545	38012
Other cows	871	963	3679	3327	2692	2543
Average number of cattle heads per holdings	0,2	0,4	1,0	2,6	6,4	17,8

Source: Census of Agriculture 2012: agriculture in Republic of Serbia, The Statistical Office of the Republic of Serbia, 2013. ISBN 978-86-6161-077-6

Table 2. Main structural indicators of agricultural holdings Republic of Serbia  
Glavni strukturni pokazatelji poljoprivrednih gazdinstava u Republici Srbiji

Livestock fund of holdings	Livestock units			
	<10	≥10-<20	≥20	Total
Livestock fund, (000) LU	1089130	266118	664641	2019889
Heads				
Bovine animals	444457	191226	272419	908102
Bovine, under 1 year old	100727	61994	99726	262447
Bovine, 1 – 2 years old	36574	27677	54532	118783
Bovine, over 2 years old	307156	101555	118161	526872
Male heads	6315	3878	5141	15334
Heifers	35119	13814	17240	66173
Dairy cows	258251	82067	90972	431290
Other cows	7471	1796	4808	14075
Average number of cattle heads per holdings	1,0	9,6	32,4	1,0

Source: Census of Agriculture 2012: agriculture in Republic of Serbia, The Statistical Office of the Republic of Serbia, 2013. ISBN 978-86-6161-077-6

Livestock unit (LU) is a standard measurement unit by which the number of heads of various species and categories is reduced to a comparable value. For calculating livestock unit used are the coefficients applicable according to the EU standards (*Handbook on implementing the FSS and SAPM definitions*, Annex 1).

### **Presence and prevalence of BVD infection among ruminants in Serbia**

As there is no BVDV control programme in Serbia and multivalent vaccines with BVDV antigen were used only recently in a few herds, the observed results reflect the natural course of the infection in the ruminant population. In the former Yugoslavia, BVDV infection was first described by Đuričković and co-workers (1966) based on clinical symptoms and pathomorphological findings, and was serologically confirmed by Cvetnić, et al. (1967). At the time, Cvetnić, et al. (1967) identified the presence of BVDV-specific antibodies using the agar-gel precipitation method in 12.67% calf sera and in 5.14% sera sampled from all animals. The first confirmation of BVDV presence was initially followed by individual small-scale studies and then by more frequent large-scale studies on the prevalence of this viral infection in Serbia. Those studies were presented in Table 3.

Following these initial investigations, this viral infection of cattle was not given much attention in Serbia. Veterinary clinicians and cattle farmers focused their attention primarily on IBR (and on other BHV1 associated infections) due to the fact that most cases were diagnosed for severe respiratory epidemic and endemic infections, particularly in large cattle feedlots during the 1970's and 1980's. Due to this problem and to the limited diagnostic methods and procedures, BVDV infection during the 1970's through part of the 1990's was a neglected infection of cattle in Serbia.

It was not earlier than the beginning of the 1990's and, most particularly, after 2000, that much more frequent, larger-scale and more thorough investigations on the presence and prevalence of BVDV infection in Serbia were initiated (Table 3.).

Jermolenko, et al. (1995) were the first in Serbia to isolate the BVD virus in 1995. A cytopathogenic (cp) strain of BVDV was isolated from the spleen and small intestine of a dead animal that showed signs of cachexia, mucosal pallor and brown diarrhoea lasting for several days. The animal originated from a herd of about 200 animals that experienced reproductive problems during the calving season.

More than five years after the first BVD virus detection in Serbia, Petrović and co-workers (2004) tested the presence of BVD virus in fetal calf serum (FCS) produced in Serbia, heparin-treated whole blood and pathological material from dead animals succumbed with BVDV suspected clinical signs in Serbia. Two positive FCS samples (ncp isolates "BVDV 0016" and "BVDV 0017") were detected in the 64 samples tested. The third positive sample was identified by examination of whole blood samples collected from an animal showing clinical signs of mucosal disease (cp isolate "BVDV Beograd"). The 3 isolates of the virus were genetically typed and subjected to phylogenetic analysis based on comparison with 15 recent Slovenian isolates and BVDV reference strains. The 5' UTR amplified by "one-tube" RT-PCR was characterised by direct sequencing and phylogenetic analysis. One isolate from Serbia (0017) was typed as BVDV 1b, and the other two (0016 and Beograd) as BVDV 1f subtype. BVDV genotype 2 isolates were not determined. Upon confirmation by the OIE Reference Laboratory for BVDV (VLA, Weybridge, UK-letter of 9 December 2002), the presence of this infection of cattle in Serbia and Montenegro was officially recognised. The Serbian BVDV isolate "Beograd" belongs to the cytopathogenic biotype of BVDV. This was the first report of a cp BVDV belonging to the 1f subtype. When the obtained results were compared to the genetic prevalence of BVDV in the rest of Europe and beyond, a matching pattern of epidemiology of BVDV was evident, even with the small number of virus isolates genotyped in that paper. Subtype 1b BVDVs have been reported from all over the



world, whereas the 1f subtype has been described so far only in Central Europe (Austria, Germany, Hungary, Italy, Slovakia, Slovenia, Serbia), plus Mozambique in cattle imported from Austria (Baule, et al., 1997). During the 1990's and in the beginning of the 21<sup>st</sup> century, cattle were not imported into Serbia (due to political embargo); therefore, it may be assumed that the BVDV subtypes described in this paper have been present for a long time in Serbia.

Except BVD virus detection, the clinical signs of the present infection was also reported in Serbia especially in the last few years. Debeljak, et al. (2009) reported a case study of mucosal disease in the field. The animal was clinically suspected of mucosal disease during routine epidemiological and clinical observation in the field. The clinical symptoms detected during the first observation included: reduced appetite, depression, heavy breathing, coughing, swelling around the eyes accompanied by excessive tear production, abundant salivation, profuse nasal discharge and elevated body temperature (41.5<sup>0</sup>C). Two days later, the following was observed: leg pain, weight bearing on certain extremities and lesions formed on the nose leather, muzzle, mucous membrane of the mouth, udder and hoof crown. The animal was sacrificed 8 days after initial symptoms of the disease. Virus isolation in the MDBK cell line showed a cytopathogenic (CP) effect after 48 hours. The isolated virus was classified as BVDV1a genotype and CP biotype, as determined by the comparison of the 5' UTR sequence with the sequence of virus representatives of some genotypes of the BVD virus and other pestiviruses (from NCBI GeneBank) as well as by the results on the virus isolation in the cell line. The nucleotide sequence of the 5'UTR region have been suggested that the virus isolate belongs to the BVDV1a group which also includes virus strain isolated from a deer (100% identical to the Deer strain).

Kurćubić and collaborators (2009; 2010a) have been tried to identify PI cattle in a few closed cattle herds in which the presence of ongoing BVD infection was confirmed by serological testing. The animals were tested by antigen ELISA test for the presence of BVDV antigen in blood sera, but PI animals was not detected. An explanation of the impossibility to detect PI experimental animals lied in the fact that testing for the presence of the virus antigen did not include sera of all age-eligible animals raised on the farm, as well as in the possibility that the spread of BVDV infection was most likely the result of the presence of acutely rather than persistently infected animals.

In attempting to find different possibilities for BVDV control in Serbia, Kurćubić and collaborators have carried out study (2011), which is aimed at evaluating the immunogenicity of two experimental inactivated (mono and polyvalent) vaccines containing bovine virus diarrhea virus (BVDV) reference and field strains. Blood sera were obtained from immunized animals (standard procedure: on days 0, 14, 28, 42 and 56 post-immunization). The monovalent vaccine showed better performance than the polyvalent one in both geometric mean titer values for induced BVDV neutralizing antibodies, as well as in the time needed for development of an immune response.

Table 3. Seroprevalence studies conducted in Serbia in the last 40 years  
Ispitivanja seroprevalence sprovedena u Srbiji tokom poslednjih 40 godina

Study No	Area of study	No analyzed animals	Tested animals	Type of cattle raising	Lab method	Results of seroprevalence studies			Reference
						% / No of seropositive animals	% / No of positive herds	% of sero-positive animals in herds	
1	Banat, Belgrade, Valjevo (north and central Serbia)	224	Dairy cattle	6 big farms*	VNT	74% (166/224)	100 % (6/6)	38.8 – 91%	Belić et al, 1973
2	Central Serbia	264	Dairy and beef cattle	2 big farms: 1 dairy and 1 beef cattle fattening farm	VNT	48.68% (129/264)	100% (2/2)	30.55 – 55.81%	Kurćubić 1993; 1995
3	Vojvodina Province, area of 7 municipalities (Northern part of Serbia)	2657 (2076 from big dairy farms, and 581 from small backyard farms**)	Dairy cattle	13 big farms and more than 100 backyard farms	VNT	50.96% (1354/2657)	84.62% (11/13) among big farms	0 – 100%	Petrović T. 2002; Petrović T. et al., 2002
4	Nis and Southern Morava areas - 9 municipalities (Southern part of Serbia)	188	Dairy cattle	Small backyard farms	ELISA	10.64% (20/188)	No data (positive herds were located in 5 out of 9 municipalities)	3.8 – 45%	Petrović M. et al, 2002
5	North Backa county in Vojvodina Province, area of 5 municipalities (Northern part of Serbia)	188 (94 from big dairy farms, and 94 from small backyard farms)	Dairy cattle	7 big farms and small backyard farms from 17 settlements in 5 municipalities	ELISA	22.34% among animals in big herds and 59.57% among animals in small herds	4/5 – 80% of big herds. No data for small herds (positive herds present on the area of all municipalities)	From 0 (1 herd) up to 71.43% in big herds No data for small herds	Molnar et al., 2003
6	Belgrade district	2473 (449 from big herds and 2024 from small backyard farms)	Dairy cattle	big farms and small backyard farms	ELISA and VNT	66.8% among animals in big herds and 4.24% among animals in small herds	No data	No data	Milošević et al., 2004
7	Vojvodina Province, area of 7 counties (Northern part of Serbia)	12083 (4506 from big herds and 7577 from small backyard farms)	Dairy cattle (12.88% of the whole cattle population on the examined area)	32 big industrial farms and small backyard farms from 16 municipalities (in total – area of 156 settlements)	VNT	38.46% (4647/12083) 62.45% among animals in big herds and 24.19% among animals in small herds	26/32 – 81% of big herds. No data for small herds (positive herds present on the area of all municipalities)	Seroprevalence in big herds ranged from 0 to 96.96% and in small herds ranged from 0-86.7%.	Petrović T., 2006
8	Central part of Serbia	100 (10 animals from 10 mini farms)	Sheep	10 mini farms	ELISA	No seropositive animals detected	All 10 examined herds were negative	/	Kurćubić et al., 2010b

\* Big commercial farms with few hundreds of lactating cows including corresponding number of heifers and calves.

\*\* Small family backyard herds, with up to 20 cows (usually 1 to 5 animals).

## Conclusion

Even though extensive surveys have been carried out, knowledge of the overall BVDV situation in Europe is still not complete. A few countries lack reports on prevalence, and representative estimates of incidence have only been reported from countries that have systematic control in place. Also, the prevalence of different genetic groups has not been estimated through formal surveys; thus, the information available is more of the type present/not present. The exact epidemiological situation of BVDV infection in Serbia is still not elucidated due to the absence of detailed serological and virus surveillance in the whole country. However, given the studies conducted so far, the extent of spread of BVDV infection in the epizootiological regions, the current health status of animals and marked clinical manifestations of the bovine disease, it is possible to assume that BVDV infection is widespread throughout the country. Except a few pilot programme for BVDV eradication on 2 or 3 farms in the past, no regional or national BVDV control programme are currently ongoing in Serbia. The obtained data and realistic assumptions on the spread of BVDV infection in Serbia, the resulting large economic losses, and the tendency to find solutions to the problem in European countries suggest the need to undertake systematic measures to check if adequate control of this viral disease of cattle could be practised in Serbia. Due to the type of cattle breeding in Serbia, it can be assumed that a combination of strict eradication programme with identification and removal of PI animals, and strict biosecurity measures in order to establish herds completely free of BVDV, that could be implemented at smaller farms (up to 50-100 cows), and BVDV infection control with removal of PI animals and with vaccination as part of the control for big farms with few hundred cows, could be the first step for BVDV control and eradication in Serbia.

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