UDC: 636.09:616.34-008.314.4:636.2
original scientific paper

Acta Agriculturae Serbica, Vol. XIV, 27 (2009) 79-92

Acta Agriculturae Serbica
FACULTY OF AGRONOMY • ČAČAK •

Identification of Cattle Persistently Infected (PI) with the Bovine Viral Diarrhoea Virus (BVDV) using a Screening Method for a Control and Eradication Programme

V. Kurćubić
Faculty of Agronomy, Čačak, Serbia

Z. Ilić
Faculty of Agriculture, ZubinPotok, Serbia

R. Đoković, S. Jevtić
Faculty of Agronomy, Čačak, Serbia

Abstract: The objective of this study was to identify PI cattle in a closed herd of a capacity of 137 dairy cows (a total of 263 animals of different age). The cattle were divided into three experimental groups. The first group comprised 15 animals tested 10-15 days before parturition. The second group was composed of 14 cows examined 10-15 days after parturition. The third group was made up of 15 cows in full lactation. Two series of blood samples were collected at an interval of 4 weeks (paired samples) from all cows from each experimental group. Blood serums taken from both series of samples were tested by an indirect enzyme immunoassay (ELISA) using the HerdChek* BVDV Ab test kit.

The results obtained in this study indicated the presence of BVD virus infection in the examined cattle. The serum samples taken on the first day of testing were seropositive in 5 animals and suspicious in 1 in the first experimental group, seropositive in 2 animals of the second group and seropositive in 3 and suspicious in 2 animals of the third experimental group.

This study is part of a project on Technological Development Ref. No. 20015 "Contemporary Tendencies in the Production, Reproduction, Disease Prevention and Health Control in Ruminants Aimed at Their Welfare and Biosafety" supported by the Ministry of Science and Technological Development of the Republic of Serbia.

The serum samples collected on day 28 tested seropositive in 5, 2 and 4 animals in the first, second and third experimental groups, respectively.

The samples of cattle that were found to be seronegative in both tests (i.e. on days 1 and 28) were tested by the enzyme immunoassay method (ELISA) using the HerdChek* BVDV Ag/Serum Plus test kit specifically designed for detecting BVD virus antigens. BVD virus antigens were not confirmed in any of the examined cattle.

Key words: BVDV, ELISA, seroprevalence, PI cattle

Introduction

Infections with bovine viral diarrhoea virus (BVDV) are 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 of the causative virus can be distinguished, BVDV-1 and BVDV-2. Their prevalences vary 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).

More recently, the BVDV-1 and BVDV-2 genotypes have been further divided into subgenotypes BVDV-1a, BVDV-1b, BVDV -2a and BVDV – 2b in North America (Ridpath *et al.* 2000, Flores *et al.* 2002). In addition, another classification depicts 11 genetic groups of BVDV (Vilcek *et al.* 2001), but it is believed today that the number is higher.

The BVDV can be also classified into biotypes – virus phenotypes, cytopathic (CP) and noncytopathic (NCP) that are based on the presence or absence of visible cytopathogenic effect (CPE) in infected cell cultures (Baker 1995).

Depending on the time of infection, there may be a significant reduction in conception rates and an increased number of abortions, malformations, stillbirths or births of persistently infected (PI) calves (Done *et al.* 1980, Mc Clurkin *et al*. 1984, Roeder *et al.*1986). PI calves are immunotolerant to the persisting virus, which is continuously propagated in their organs and tissues. As these animals constantly shed the virus, they are key to the spread of BVDV and consequently their detection plays a pivotal role in any control or eradication programme. Many PI animals die of mucosal disease (MD), but more often they leave the herd at an early age due to other complications. However, a few may survive for many years (Houe 1993, Roeder and Drew 1984).

The first BVDV control and eradication programmes were launched in 1993-1994 in the Scandinavian countries – Denmark, Finland, Norway and Sweden – and they primarily relied on detection and removal of PI animals from a herd (Waage *et al*. 1994). The Norwegians used an indirect ELISA to test bulk milk samples collected from 26,430 dairy herds. Other Scandinavian countries followed the example of Norway. The programme included: 1) identification of virus-free herds, 2) prevention of infection in these herds and 3) reduction in the number of infected herds. A reduction in the number of infected herds was achieved through detection and removal of PI animals from herds, as well as through prevention of acute infection (Oirschot *et al.* 1999, Bitsch and Ronsholt 1995).

Roeder and Harkness (1986) suggested that the detection and removal of PI animals was of paramount importance in BVD control. This also involves testing of all animals in a herd for the presence of the virus, which is a rather expensive procedure (Baker 1987).

As reported by Irene Greiser-Wilke (2003), the aim of control programmes in high density areas with high seroprevalence is to minimize economic losses by reducing the incidence of PI animals and thereby virus circulation (German model). A herd is declared BVDV unsuspicious if all animals of up to 36 months are BVDV antigen negative.

Waage *et al*. (1996) have suggested that herds are declared free of BVD virus infection if two successive serologically negative tests are obtained, the first one for bulk tank milk samples, bulk milk of first-calving cows and collected serum samples from young animals, and the second one for the collected serum samples from young animals tested at least 4 months later.

Bitsch and Ronsholt (1995) have reported in their study that elimination of PI animals may result in eradication of BVDV infection. Upon eradication, herds can be declared free of infection if 3 calves of over 8 months of age test negative for the presence of specific antibodies and virus in a blood sample during a testing period of 12 months or more after the removal of PI animals.

Upon removal of PI animals, particular attention should be given to the introduction of new animals into a herd. These animals should be tested for the presence of the virus, and if they are pregnant, their new-born calves should be examined at birth by blood sampling before colostrum feeding for detection of virus infection. After adequate steps have been taken, focus should be placed on the prevention of the incidence of transplacental infection (Baker 1987).

Major risk factors for the spread of the BVD virus infection include trading and other movement of animals between herds, live attenuated vaccines, semen and embryos. Prevention of virus infection by semen is achieved through regular serological testing of seronegative bulls and/or testing of semen from all seropositive bulls (Lindberg and Alenius 1999). EU regulations on artificial insemination centres envisage stringent BVD virus infection control procedures for bulls (Bitsch and Ronsholt 1995). Embryos pose danger if they are not washed adequately, donors being either PI or acutely infected animals. Another constant risk comes from attenuated vaccines due to frequent contamination with both BVD viral genotypes (Lindberg and Alenius 1999).

Material and Methods

Experimental Animals

A study was conducted at the Farmad dairy farm located at the village of Vrdila near Kraljevo to assay the serological status of experimental animals (cattle) and identify persistently infected (PI) animals. The total capacity of the farm was 137 dairy cows, but it housed 263 animals of all categories. All animals were of Simmental breed (Bavarian Simmental cattle – 150 late-gestation heifers were imported at the end of 2005), and the farm was filled to capacity with replacement

animals from its own herd. The cattle were raised in a closed herd – the breeding material was not obtained through purchase.

A total of 44 experimental animals of different age (born during the period 2003-2006) were selected and divided into three experimental groups. The Experimental Group I comprised 15 animals tested at 10-15 days before parturition. The Experimental Group II was composed of 14 cows examined 10-15 days after parturition. The Experimental Group III was made up of 15 cows in full lactation.

The above classification scheme was accepted since blood samples were taken from the experimental animals for simultaneous testing within the part of the project No. 20015 focused on determining metabolic profiles in dairy cows, which necessitated examination of cattle at different periods of metabolic activity. The information obtained on the cattle selected for the trial included identification, dam ID numbers, sire ID numbers, date of birth, productivity, artificial insemination of cows and heifers and date of last parturitions.

The Results section provides information on treatments, incidence of diarrhoea, placental retentions, abortions and reproduction disorders. Sources of information included the master registry, ambulance protocols and artificial insemination record book.

Sampling of material

Blood samples were taken from the caudal vein (v. coccygica) of all examined cattle (n = 44) by means of vacutaineers and sterile needles for each animal. To test paired serum samples, after the first day (beginning) of the trial, the second blood sampling was conducted on day 28 of the trial. The collected blood samples were left to coagulate spontaneously at room temperature. The obtained blood sera were drained and centrifuged at 3,000 revolutions/minute. The separated sera were transferred into sterile vials using pipettes and stored at -20^0C until assayed.

Screening methods

ELISA test was used to detect specific anti-BVDV antibodies and prove the presence of BVD antigens in the blood sera of the tested animals. The results were read using an ELISA KINETIC-QCL Reader (BIO-WHITTAKER, Austria).

Indirect ELISA test

Testing of the blood sera of the experimental cattle (cows) for the detection of specific anti-BVD antibodies was conducted using a commercial indirect ELISA Antibody Test Kit HerdChek*BVDV Ab, (IDEXX Laboratories Inc.)

HerdChek* BVDV-Ab is an indirect enzyme immunoassay designed to detect BVDV antibodies in serum, plasma and milk samples from bovines. BVDV antibodies of the sample are bound to the antigen on the microtitration plates. After incubation of the test sample in the well, captured BVDV antibodies are detected by anti-bovine peroxidase conjugate. Next, the unbound conjugate is washed away and a

substrate/chromogen solution required for visualization i.e. reading of the test reaction is added. The substrate is converted into a product which reacts with the chromogen to generate a blue colour. A stop solution is, finally, added to terminate the above enzymatic reaction.

Test results were obtained by spectrophotometry based on the absorption of the sample using a 450 nm filter. The sample to positive ratio was calculated by the absorbance [A(450)] obtained with the test sample and a positive control, corrected for the absorbance of the negative control. Colour development indicates the presence of BVDV antibodies in the test sample (positive results).

Elisa test for the detection of BVDV antigens

Testing of the blood sera of the experimental cattle (cows) for the detection of BVDV antigens was conducted using Herd Chek* BVDV Ag/Serum Plus (IDEXX Laboratories Inc.), a commercial enzyme immunoassay (ELISA).

HerdChek BVDV-Ag/Serum Plus is an enzyme immunoassay designed to detect BVDV antigens in serum, plasma, whole blood and ear notch tissue. A microtitration format has been configured by immobilizing specific monoclonal antibodies for BVDV (E^{ms}) on the plates. After incubation of the test sample in the well, BVDV Ag on the plates is detected by specific antibodies and a peroxidase conjugate. The unbound conjugate is washed away and a substrate/chromogen solution required for visualization i.e. reading of the test reaction is added. In the presence of the enzyme, the substrate is converted into a product which reacts with the chromogen to generate a blue colour. A stop solution is, finally, added to terminate the above enzymatic reaction. Test results were obtained by spectrophotometry based on the absorption of the sample using a 450 nm filter. The corrected OD values of the samples are calculated by the absorbance [A(450)] obtained with the test sample and corrected for the absorbance of the negative control.

Results and Discussion

The results of the study are given in tabular form (Tables 1-4) and are commented upon below the tables.

The samples taken from the Experimental Group I of 15 cows tested on day 10 to day 15 before parturition (ante partum) were seropositive in 5 animals on both day 1 and day 28 of testing (the cows Nos. 07502, 18540, 37531, 70168 and 07504) and suspicious and seronegative in cow No. 84489 on day 1 and day 28, respectively (Table 1). Accordingly, there were 5 seropositive experimental animals on both day 1 and day 28 of testing (33.33 %).

During the sampling of material from quarantined cows being prepared for parturition, animal No. 70168 was identified as being sick with profuse watery diarrhoea that started the previous day, mastitis, inappetence, hunching posture and general deterioration. The collected anamnestic data showed that the diseased animal had been subjected to (antibiotic, supportive, infusion) treatment since the day before. The animal was found to be seropositive on both day 1 and day 28 of testing. It was cured, but its delivery (on 15 September 2008) was

followed by the retention of placenta, which was removed manually, which was indicative of possible consequences of BVDV infection.

Tab. 1. Testing for the presence of antibodies in the Experimental Group I cows on day 10 to day 15 before parturition (ante partum)

No.	Designation	ELISA S/P Day 1 of testing	ELISA S/P Day 28 of testing	ELISA +/- Day 1 of testing	ELISA +/- Day 28 of testing
1.	8420	0.167	0.235	-	-
2.	1699	0.122	0.132	-	-
3.	33129	0.112	0.121	-	-
4.	07502	1.097	1.101	+	+
5.	84489	0.359	0.215	S	-
6.	18540	1.103	0.886	+	+
7.	64585	0.242	0.296	-	-
8.	79571	0.160	0.270	-	-
9.	37531	1.104	0.694	+	+
10.	32038	0.166	0.127	-	-
11.	13868	0.179	0.311	-	-
12.	30045	0.308	0.146	-	-
13.	70168	1.246	1.409	+	+
14.	02619	0.167	0.145	-	-
15.	07504	0.742	0.617	+	+
	t_{exp}		ns		

* Positive control mean (POS) 0.956, negative control mean (NEG) 0.153, difference 0.803

The obtained health data showed that placental retention in 2008 was recorded in animals Nos. 07502, 64585, 79571, 32038, 13868, 30045 and 70168. Reproduction disorders were recorded in animals Nos. 33129, 84489, 18540, 37531, 32038 and 30045. Outbreaks of abortions were not reported for the Experimental Group I cattle.

The samples taken from the Experimental Group II of 14 cows tested on day 10-15 after parturition (post partum) were serpositive in two animals (14.28% - Nos. 36875 and 63890) on both day 1 and day 28 of testing (Table 2).

The obtained health data showed that placental retention in 2008 was recorded in animals Nos. 37656, 19345, 36875 and 63890. Outbreaks of diarrhoea and abortions were not reported for the Experimental Group II cattle.

Tab. 2. Testing for the detection of antibodies in the Experimental Group II of cows on day 10-15 after parturition (post partum)

No.	Designation	ELISA S/P Day 1 of testing	ELISA S/P Day 28 of testing	ELISA +/- Day 1 of testing	ELISA +/- Day 28 of testing
1.	27208	0.138	0.118	-	-
2.	37656	0.155	0.147	-	-
3.	4605	0.136	0.127	-	-
4.	1661	0.156	0.164	-	-
5.	19345	0.113	0.120	-	-
6.	76466	0.144	0.143	-	-
7.	4634	0.155	0.163	-	-
8.	39246	0.180	0.180	-	-
9.	73573	0.123	0.141	-	-
10.	1700	0.158	0.121	-	-
11.	54882	0.156	0.144	-	-
12.	73245	0.279	0.240	-	-
13.	36875	0.935	1.025	+	+
14.	63890	1.203	1.167	+	+
	t_{exp}	ns			

* Positive control mean (POS) 0.956, negative control mean (NEG) 0.153, difference 0.803

Three seropositive animals (33.33% - Nos. 01240, 84747 and 73512) and two suspicious animals (13.33% - Nos. 37134 and 76460) were detected on the first day of testing in the Experimental Group III of 15 dairy cows in full lactation. In the repeated testing of samples collected on day 28, four seropositive dairy cows were detected (26.66% - Nos. 01240, 84747 and 73512 and cow No. 16542 which was not seropositive on the first day of testing) and the dairy cows that were previously found suspicious (Nos. 37134 and 76460) were confirmed seronegative (Table 3).

The obtained health data showed that placental retention in 2008 was reported for animals Nos. 6462, 20514, 84747, 02618, 25794 and 65835. Outbreaks of diarrhoea were not reported for the experimental Group III cattle. Outbreaks of abortions were reported for the animal No. 76462 on 27 March 2008.

Statistical data analysis using an individual t-test (SPS statistical software ver. 5.0) suggested that the 3 experimental groups exhibited no statistically significant differences between the ELISA S/P values obtained on day 1 and those obtained on day 28 of the animal testing.

In overall terms (including 44 experimental animals from all 3 experimental groups), out of 11 animals (25%) that tested seropositive, 10 were seropositive on both day 1 and day 28 of the testing and only one animal of the Experimental Group III (No. 16542) was seropositive on day 28 of the testing, being a sure indication of the presence of infection.

These results are not surprising and they conform with the published findings of other authors, since seroprevalence may vary widely.

Tab. 3. Testing for the detection of antibodies in the Experimental Group III of cows in full lactation

No.	Designation	ELISA S/P Day 1 of testing	ELISA S/P Day 28 of testing	ELISA +/- Day 1 of testing	ELISA +/- Day 28 of testing
1.	16542	0.126	1.311	-	+
2.	61038	0.178	0.201	-	-
3.	01240	0.808	0.724	+	+
4.	37134	0.386	0.148	S	-
5.	76462	0.212	0.173	-	-
6.	20514	0.211	0.142	-	-
7.	84747	1.168	1.085	+	+
8.	76460	0.376	0.187	S	-
9.	73512	1.273	1.324	+	+
10.	02618	0.163	0.133	-	-
11.	93953	0.243	0.182	-	-
12.	05036	0.113	0.150	-	-
13.	25794	0.160	0.124	-	-
14.	16161	0.206	0.140	-	-
15.	65835	0.223	0.161	-	-
	t_{exp}		ns		

* Positive control mean (POS) 0.956, negative control mean (NEG) 0.153, difference 0.803

In his M. Sc. thesis, Petrovic (2002) conducted a thorough research on the presence of serum-neutralizing (SN) BVDV antibodies in the blood sera of cattle of over 6 months of age raised in the South Bačka and Srem Districts. The testing included 2,657 samples of blood sera, 1,354 (50.95%) of which were found to be positive. BVDV infection was detected in the whole area that was epizootiologically investigated.

Molnar *et al.* (2003) identified serologically positive animals in all 5 investigated municipalities of the North Bačka District. Investigations conducted by Petrović M. M. *et al.* (2002) in the Niš and South Morava regions, which included a lower number of animals raised in 9 municipalities, determined a serological response to the BVDV virus in animals from 5 municipalities. The percentage of serologically positive animals ranged from 3.8 % to 45 %.

Investigations conducted previously at two cattle farms showed that the percentage of seropositive animals at a dairy farm with 178 cows ranged from 30.55 % to 52.24 %, depending on cow age. Investigations conducted at a farm producing beef cattle (aged 6 to 7 months) suggested that 48 or 55.81% of the 86 sera tested were seropositive (Kurćubić 1993).

Identification of persistently infected (PI) animals among the animals that tested seronegative in both tests (conducted on day 1 and day 28) in the first testing was conducted by ELISA (an enzyme immunoassay designed to detect BVDV antigens in serum, using a test/kit HERDCHEK* BVDV Ag/Serum Plus). The te-

sting included 30 animals (blood sera) and the virus antigen was not detected in any sample, all S-N values being negative (Table 4).

Tab. 4. Detection of animals persistently infected (PI) with the BVD virus in animals that tested seronegative in both tests (on days 1 and 28) in the first trial by testing blood sera using BVDV Ag ELISA

no	Designation	S-N values	+/-
1.	33129	0.172	-
2.	1699	0.181	-
3.	8420	0.199	-
4.	64585	0.163	-
5.	32038	0.204	-
6.	02619	0.191	-
7.	13868	0.187	-
8.	79571	0.192	-
9.	30045	0.156	-
10.	73245	0.165	-
11.	19435	0.199	-
12.	27208	0.150	-
13.	4634	0.181	-
14.	4605	0.181	-
15.	1661	0.194	-
16.	1700	0.177	-
17.	39246	0.152	-
18.	73573	0.162	-
19.	76466	0.185	-
20.	37656	0.136	-
21.	54882	0.168	-
22.	16161	0.210	-
23.	20514	0.183	-
24.	76462	0.211	-
25.	61038	0.148	-
26.	25794	0.161	-
27.	93953	0.194	-
28.	65835	0.147	-
29.	02618	0.180	-
30.	05036	0.184	-
31.	Nc	0.201	
32.	Pc	1.466	
	* POS 1.466	NEG 0.201	1.265

An explanation of the impossibility to detect PI experimental animals lies 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 well-known fact that the prevalence of PI animals is extremely low (0.75%-2%).

Houe *et al.* (2003) have reported that PI animals can be found in about 50 percent of all herds in countries where systematic control of BVDV infection has not been established.

Billinis *et al.* (2005) tested 39 herds (a total of 6,333 cows) in Greece within a volunteer BVD eradication programme based on the detection of PI animals. The objective of their study was to estimate the prevalence of BVD-antigen positive and PI animals and investigate the significance of relationship between the estimated prevalence and herd size. The animals were tested by antigen ELISA. The second sample was collected from positive animals after a three-week period. The animals that tested positive again in the second testing were classified as PI. Antigen positive and PI animals were detected in all herds. The mean prevalence of PI animals was 1.3% (0.8-1.8%). Herd size was not related to the prevalence of antigen-positive or PI animals.

In Belgium, Letellier *et al.* (2005) used a BVD-antigen ELISA test as a strategy to detect PI animals in animals purchased from commercial suppliers with the aim to reduce BVDV circulation. They also used a combination of PCR analysis on pooled blood samples and BVD-antigen ELISA on individual blood samples in cases of clinical suspicion. Less than 4% of purchased animals were tested in 2003. The estimated prevalence of BVD-antigen positive animals was 1% in the Northern part and 0.75% in the Southern part of the country. However, the confirmation of persistent viremia was performed only in a limited number of antigen-positive animals.

In Turkey, Ozkul *et al.*(1995) determined animals persistently infected with pestivirus at rates of 0.5%, 0.12% and 0.12% respectively in 3 of 5 dairy farms.

There is a need for a continued study on identifying PI animals by testing all animals above 4 months of age in the herd. A cost-effective testing method has been also proposed by other authors. Deregt and Loewen (1995) suggest that a more practical solution would be to, firstly, perform serological testing of all animals in a herd by a serum-neutralizing (SN) test and then to identify the virus in animals that have no or low SN antibody titers. PI animals are viremic and, generally, seronegative. They can be seropositive in cases such as maternal immunity in young animals (therefore, animals above 4 months of age are tested) and low SN antibody against heterologous BVD strains.

The presence of viremia in such animals requires confirmation, after a repeat testing to be conducted 3-4 weeks later, if they should be declared PI. If viremia is not confirmed by repeat testing, the animal should be considered acutely, rather than persistently infected and should not be culled.

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
c) surveillance to monitor the progress of interventions and to rapidly detect new infections.

Conclusions

The samples taken from the Experimental Group I of 15 cows tested on day 10 to day 15 before parturition (ante partum) were seropositive in 5 animals on both day 1 and day 28 of testing (the cows Nos. 07502, 18540, 37531, 70168 and 07504) and suspicious and seronegative in cow No. 84489 on day 1 and day 28, respectively (Table 1). Accordingly, there were 5 seropositive experimental animals on both day 1 and day 28 of testing (33.33 %).

The samples taken from the Experimental Group II of 14 cows tested on day 10-15 after parturition (post partum) were seropositive in two animals (14.28% - No. 36875 and 63890) on both day 1 and day 28 of testing

Three seropositive animals (33.33% - Nos. 01240, 84747 and 73512) and two suspicious animals (13.33% - Nos. 37134 and 76460) were detected on the first day of testing in the Experimental Group III of 15 dairy cows in full lactation. In the repeated testing of samples collected on day 28, four seropositive dairy cows were detected (26.66% - Nos. 01240, 84747 and 73512 and cow No. 16542 which was not seropositive on the first day of testing) and the dairy cows that were previously found suspicious (Nos. 37134 and 76460) were confirmed seronegative.

In overall terms (including 44 experimental animals from all 3 experimental groups), out of 11 animals (25%) that tested seropositive, 10 were seropositive on both day 1 and day 28 of the testing and only one animal of the Experimental Group III (No. 16542) was seropositive on day 28 of the testing.

ELISA, an enzyme immunoassay designed to detect BVD antigens in serum, and a test/kit HERDCHEK* BVDV Ag/Serum Plus were used to detect persistently infected animals (PI) among cattle that were seronegative in both tests (on day 1 and day 28) in the first testing. A total of 30 animals (blood sera) were tested, and the virus antigen was not confirmed in any of the test animals, all S-N values being negative.

References

Baker J.C. (1987): Bovine viral diarrhea virus: A review*; JAVMA*, **190** (**11**): 1449-1458.

Baker J.C. (1995): The clinical manifestation of bovine viral diarrhea infection. *Vet. Clin. N. A. Food Anim. Pract.,* **11**: 425-445.

Billinis C., Leontides L., Amiridis G.S., Spyrou V., Kostoulas P., Sofia M. (2005): Prevalence of BVDV infection in greek dairy herds. *Preventive veterinary medicine*, **72 (1-2)**: 75-79

Bitsch V., Ronsholt L. (1995): Control of bovine viral diarrhea virus infection without vaccines. *Veterinary Clinics of North America - Food Animal Practice*, **11(3):** 627-640.

Deregt D., Loewen K.G. (1995): Bovine viral diarrhoea virus: Biotypes and disease; *Can. Vet. J.*, **36**: 371-378.

Done J.T., Terlecki S., Richardson C., Harkness J.W., Sands J.J., Patterson D.S., Sweasey D., Shaw I.G., Winkler, C.E., Duffell S.J. (1980): Bovine virus diarrhoea – mucosal disease virus: pathogenicity for the fetal calf following maternal infection. *Vet. Rec*., **106 (23)**: 473-479.

Flores, E.F., Ridpath J.F., Weiblen R., Vogel F.S.F., Gil Laura H.V.G. (2002): Phylogenetic analysis of Brazilian bovine viral diarrhea virus type 2

(BVDV-2) isolates: evidence for a subgenotype within BVDV-2. *Virus Research,* **87**: 51-60.

Greiser-Wilke I., Wilke W.B., Moennig V. (2003): Bovine viral diarrhoea eradication and control programmes in Europe. Detecting and Controling BVDV Infections. *Biologicals*, **31(2)**: 113-118.

Houe H., Pedersen K.M., Meyling A. (1993): A computerized spreadsheet model for calculating total annual losses due sensitivity analysis of selected parameters. *In Proc. 10th International Symposium for Veterinary Epidemiology and Economics, 17-21 November, Vina del Mar, Chile.* International Society for Veterinary Epidemiology and Economics (available at: www.sciquest.org.nz).

Houe H. (1993): Survivorship of animals persistently infected with bovine virus diarrhea virus (BVDV). *Prev. vet. Med*., **15 (4)**: 275-283.

Houe H., Kjer Ersbøll A., Lindberg A. (2003): Metaanalysis for establishing the association between prevalence of infection with bovine virus diarrhea virus (BVDV) and demographic risk factors. *In Proc. 10th International Symposium for Veterinary Epidemiology and Economics, 17-21 November,* Viña del Mar, Chile. International Society for Veterinary Epidemiology and Economics (www.sciquest.org.nz).

Kurćubić V. (1993): *Serološka ispitivanja goveda na infekciju virusom bovine virusne dijareje.* Magistarski rad, Veterinarski fakultet Univerziteta u Beogradu, Katedra za Mikrobiologiju i imunologiju, Beograd.

Lindberg A.L.E., Alenius S. (1999): Principles for eradication of bovine viral diarrhoea virus infections in cattle populations. *Veterinary Microbiology,* **64**: 197-222.

Lindberg A., Brownlie J., Gunn G.J., Houe, H., Moennig V., Saatkamp H.W., Sandvik T., Valle P.S. (2006): The control of bovine viral diarrhoea virus in Europe: today and in the future. *Rev. Sci. Tech. Off. Int. Epiz*., **25 (3)**: 961-979.

Letellier C., De Meulemeester L., Lomba M., Mijten E., Kerkhofs P. (2005): Detection of BVDV persistently infected animals in Belgium: Evaluation of the strategy implemented. *Preventive Veterinary Medicine,* **72**: 121–125.

Mc Clurkin A.W., Littledike E.T., Cutlip R.C., Frank G.H., Coria M.F., Bolin S.R. (1984): Production of cattle immunotolerant to bovine viral diarrhea virus. *Can. J. Comp. Med*., **48 (2)**: 156-161.

Molnar T., Kiškarolj F, Molnar O. (2003): Bovina virusna dijareja – prva serološka ispitivanja na epizootiološkom području VSI „Subotica". *„5. Jugoslovenski Epizootiološki dani",* Palić, Subotica, Zbornik referata i kratkih sadržaja, 56-59.

Oirschot J.T., Bruschke C.J.M., van Rijn P.A. (1999): Vaccination of cattle against bovine viral diarrhoea. *Veterinary Microbiology*, **64**: 169-183.

Özkul, A., Çabalar, M., Bilge, S., Akça, Y., Burgu, I. (1995): Süt sığırciliği işletmelerinde rastlanan IBR/IPV ve BVD virus enfeksiyonlannin infertilite olgulanndaki rolü. *Ankara Üniv, Vet. Fak. Derg.,* **42**: 381-387.

Petrović M.M., Đuričić Bosiljka, Ignjatović R., Stojanović G., Petreski I. (2002): Ispitivanje raširenosti virusne dijereje goveda/bolesti sluzokoža (BVD/MD) ELISA testom kod mlečnih krava Niškog i južnomoravskog epizootiološkog područja. *„IV Jugoslovenski Epizootiološki dani"*, Mataruška Banja, Zbornik referata i kratkih sadržaja, 224-226.

Petrović T. (2002): *Ispitivanje raširenosti infekcije izazvane virusom goveđe dijareje (BVD) kod priplodnih goveda.* Magistarska teza, Fakultet veterinarske medicine, Univerzitet u Beogradu, Katedra za zarazne bolesti životinja i bolesti pčela.

Ridpath J.F., Neill, J.D., Frey, M. (2000): Phylogenetic antigenic and clinical characterization of type 2 BVDV from North America. *Vet. Microbiol,*. **77**: 145-155.

Ridpath J.F. (2005): Practical significance of heterogeneity among BVDV strains: impact of biotype and genotype 1 and genotype 2 BVDV. *Virology*, **212 (1)**: 259-262.

Roeder P.L., Drew T.W. (1984): Mucosal disease of cattle: a late sequel to fetal infection. *Vet. Rec.,* **114 (13)**: 309-13.

Roeder P.L., Harkness J.W. (1986): Prospects for control. *Veterinary Record*, **118**: 143-147.

Roeder P.L., Jeffrey M., Cranwell M.P. (1986): Pestivirus fethopathogenicity in cattle: changing sequale with fetal maturation. *Vet. Rec*., **118 (2)**: 44-48.

Vilcek S., Paton D.J., Durkovic B., Strojny L., Ibata G., Moussa A., Loitsch A., Rossmanith W., Vega S., Scicluna M.T., Palfi V. (2001): Bovine viral diarrhea virus genotype 1 can be separated into at least eleven genetic groups. *Arch. Virol,*. **146**: 99-115.

Vilcek S., Durkovic B., Kolesarova M., Paton D.J. (2005): Genetic diversity of BVDV: consequences for classification and molecular epidemiology. *Prev. vet. Med*., **72 (1-2)**: 31-35.

Waage S., Krogsrud J., Nyberg O. (1994): The Norwegian programme for eradication of bovine viral diarrhoea/mucosal disease. Proc. *18th World Buiatrics Congress:* 26th Congress of the Italian Association of Buiatrics, Bologna, Italy, 773-776.

Waage S., Krogsrud J., Nyberg O., Sandvik T. (1996): Result achieved by a national programme for the eradication of bovine virus diarrhoea, *In:* Edwards S, Paton DJ, Wensvoort G. (eds)., Proc. *Third ESVV Symposium on Pestivirus Infections,* 19-20 September 1996, Lelystad, The Netherland, Central Veterinary Laboratory, 170-172 pp.

IDENTIFIKACIJA PERZISTENTNO INFICIRANIH ŽIVOTINJA (PI) VIRUSOM GOVEĐE VIRUSNE DIJAREJE (BVDV) SKRINING METODOM U CILJU KONTROLE ZAPATA I PROGRAMA ERADIKACIJE

- originalni naučni rad -

V. Kurćubić
Agronomski fakultet, Čačak

Z. Ilić
Poljoprivredni fakultet, ZubinPotok

R. Djoković, S. Jevtić
Agronomski fakultet, Čačak

Rezime

Cilj našeg rada je bio identifikacija PI goveda u zapatu zatvorenog tipa, kapaciteta 137 muznih grla (ukupno 263 grla različitih starosnih kategorija). Formirali smo 3 ogledne grupe, od kojih je prvu činilo 15 grla na 10-15 dana do telenja. Drugu grupu je činilo 14 plotkinja, koje su bile 10-15 dana posle telenja. Treću oglednu grupu je činilo 15 grla, koja su bila u punoj laktaciji. Od svih grla, iz sve 3 ogledne grupe, uzeta je krv dvokratno (u intervalu od 4 nedelje – parni uzorci). Ispitivanje krvnih seruma je vršeno za obe serije uzoraka indirektnom imunoenzimskom probom (ELISA), uz pomoć test kita HerdChek* BVDV Ab.

Dobijeni rezultati su ukazali na prisustvo infekcije virusom BVD kod ispitivanih grla. U uzorcima seruma uzetim prvog dana ispitivanja u prvoj oglednoj grupi utvrđeno je 5 seropozitivnih grla i jedno sumnjivo, u drugoj oglednoj grupi utvrđena su 2 seropozitivna grla, a u trećoj 3 seropozitivna i dva sumnjiva grla. U uzorcima seruma uzetim 28. dana ispitivanja u prvoj oglednoj grupi utvrđeno je 5 seropozitivnih grla, u drugoj oglednoj grupi utvrđena su 2 seropozitivna grla, a u trećoj 4 seropozitivna grla.

Uzorci seruma grla koja su bila utvrđena kao seronegativna u oba ispitivanja (prvog i 28. dana) ispitani su metodom enzimskog imunoispitivanja (ELISA), uz pomoć test kita HerdChek* BVDV Ag/Serum Plus, koji je pripremljen da može utvrditi antigene virusa BVD. Ni kod jednog od ispitivanih grla nije utvrđeno prisustvo antigena virusa BVD.