

## Influence of category, herd size, grazing and management on epidemiology of bovine viral diarrhoea in dairy herds

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### Abstract

The aim of this study was to estimate the influence of category, herd size, common grazing and management as risk factors in maintaining bovine viral diarrhoea infection in dairy herds. A total of 987 sera samples obtained from 202 heifers, 653 cows and 132 calves from 103 herds in Croatia were examined by enzyme-linked immunosorbent assay. In order to establish the prevalence of persistently infected cattle, 35 herds were selected. Ear notch tissue samples from all animals in selected herds ( $n = 2284$ ) were collected and analyzed by antigen enzyme-linked immunosorbent assay. The true prevalence of specific antibodies was 61.61% and the estimated prevalence of exposure to bovine viral diarrhoea virus at the herd level was 100%. The prevalence of persistently infected animals was 0.53% and the prevalence of persistently infected herds was 20%. The antibodies prevalence was higher in cows, in herds that use common pasture and in larger herds ( $P < 0.001$ ). The prevalence of persistently infected animals was not connected with the herd size but production management on big farms contributed to maintaining the virus. The obtained results suggest that production management was an important risk factor in bovine viral diarrhoea epidemiology. High prevalence of antibodies and high prevalence of persistently infected herds requires implementation of control and eradication programs at a national or even regional level. The presented data complete the BVD epidemiological investigations from this part of Europe.

*BVDV, epidemiology, risk factors*

Bovine viral diarrhoea virus (BVDV) belongs to the Pestivirus genus within the *Flaviviridae* family (Heinz et al. 2000). The Pestivirus genus consists of four closely related species: Bovine viral diarrhoea virus 1 (BVDV-1), Bovine viral diarrhoea virus 2 (BVDV-2), Classical swine fever virus (CSFV) and Border disease virus (BDV). Both BVDV species occur in cytopathic (cp) and non-cytopathic (ncp) forms according to whether or not it produces visible change in cell cultures. Bovine viral diarrhoea virus is a causative agent of disease in domestic and wild ruminants. Breeds of cattle do not have any influence on BVD infection (Brock et al. 2005). In contrast, the influence of herd size measured as the number of cows, mean distance to neighbouring herds, and sharing of common pasture are proven as important risks factors in BVD epidemiology (Valle et al. 1999; Alban et al. 2001).

Persistently infected (PI) cattle act as a viral reservoir and main factor of virus maintenance in the herds (Houe 1999). Infection with ncp biotypes in immunocompetent and seronegative cows or heifers in the first four months of pregnancy may lead to the birth of PI calves with no apparent clinical signs.

Some European countries conduct a national program for control and eradication of BVD. Before starting the program, in most European countries the disease was present in the endemic form with a prevalence of specific antibodies of 60–80%, and with a prevalence of PI cattle of 0.5–2% (Lindberg et al. 2006; Robesova et al. 2009). In some countries (Norway), the program has been successfully completed, and in some countries it is in its final stage.

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Different diagnostic methods can be used for the diagnosis and monitoring. However, Enzyme-Linked Immunosorbent Assay (ELISA) and antigen ELISA are the best tools for screening because they are simple, inexpensive and fast methods with high sensitivity and specificity (Hill et al. 2007). The virus can be detected in almost all organs of PI animals (Houe 1999). The advantage of ear notch tissue samples is in easier sampling. Also, the presence of antibodies does not interfere with virus detection.

In Croatia, there is no national program for control and eradication of BVD, and vaccination is conducted just in some herds on voluntary basis. According to the latest survey, the prevalence of specific antibodies was 79.2% (Biuk-Rudan et al. 1999). However, in that research only 124 cattle were tested in four herds.

The aim of this study was to estimate the influence of category, herd size, common grazing and production management as risk factors in maintaining BVD infection within dairy herds. Furthermore, this study should elucidate the role of BVD in dairy herds and provide information on the BVD situation in Croatia, and provide all necessary data as basis for the selection of control and eradication strategy.

### Materials and Methods

#### Study populations

Stratified random sampling was used for selection of non-vaccinated dairy herds and animals in herds. Dairy herds were randomly selected based on the number of dairy herds from the Unique Register of domestic animals. At the end of 2008, 184 692 dairy cattle and 22 902 herds were recorded in the register; constituting 82.22% of all cattle population in Croatia. Animals used in this study were randomly selected based on the list of animals for each herd from the register using a computer generated list. Sample size required for estimation of prevalence of antibodies and prevalence of PI cattle were calculated using WIN Episcope version 2.0 (EPIDECON). According to this program we needed to test 103 herds if estimated antibody prevalence was 50% to accomplish confidence of 95% within 10%. For PI cattle prevalence, according to the same program we needed to test 35 herds if estimated prevalence of PI cattle was 10% to accomplish confidence of 95% within 10%. Furthermore, to establish antibody prevalence with the estimated prevalence of 50% we needed to test at least four animals within a herd to detect at least one positive animal. For PI animal detection in herds with the estimated prevalence of 0.5% we needed to test all animals to accomplish detection of at least one positive animal.

Depending on sizes, herds were classified into four categories: I - herds with up to 20 animals, II - herds with 21–50 animals, III - herds with 51–100 animals and IV - herds with 101 and more animals. For antibody prevalence study, 103 herds were selected including herds that use common pastures. The sizes of selected herds were as follows: 36 herds with up to 20 animals, 25 herds with 21–50 animals, 30 herds with 51–100 animals, 12 herds with 101 and more animals. The samples were collected from the whole counties except for the Dubrovnik County.

For PI cattle prevalence, 35 herds were selected. The sizes of selected herds were as follows: 13 herds with up to 20 animals, 7 herds with 21–50 animals, 9 herds with 51–100 animals, 6 herds with 101 and more animals. All samples for PI cattle prevalence study (n = 2284) originated from herds that do not use common pastures.

#### Sampling

Sera samples were obtained from 202 heifers, 653 cows and 132 calves within 103 herds between January 2009 and January 2010. Blood samples were collected from the tail vein into Vacutainer systems. At least 5 ml per animal was collected. Blood samples were centrifuged for 10 min at 200 × g. Sera were placed into sterile tubes and stored at –20 °C until testing. The minimum number of collected sera per herd was 9 and the maximum was 11. At the same time the blood samples were collected, data on the category, herd size and data considering the use of common pasture were collected. During sampling for PI prevalence, descriptive data on the organisation of production (management) on farms were collected.

Ear notch tissue samples (n = 2284) for virus prevalence study were collected using piglet ear-notching pliers.

#### Serology

The test to detect BVDV antibodies was performed using a commercially available kit according to manufacturer's instructions, in which wells were coated with BVDV antigen (Herdchek BVDV, IDEXX, Liebefeld-Bern, Switzerland). The true antibody prevalence at individual level was calculated from apparent according to following formula: (apparent prevalence + Ag ELISA specificity – 1) / (Ag ELISA sensitivity + Ag ELISA specificity – 1).

#### Persistent infection

For detection of persistently infected animals, viral antigen was detected in ear notch tissue using a commercially available kit according to manufacturer's instructions, in which wells are coated with BVDV E<sup>ms</sup> antibody (Herdchek BVDV Ag/Serum Plus, IDEXX, Liebefeld-Bern, Switzerland). From all virus positive cattle

at initial sampling, ear notch tissue sampling was repeated after 4 weeks. Persistent infection was confirmed with repeated positive results 4 weeks after first testing.

#### Data analysis

For statistical analysis, Stata 10 (Statacorp, Texas, USA) program was used. Prevalence of specific antibodies among different categories and herds of different sizes were compared using Chi square test. Using the same test antibodies, prevalence was compared between herds that do not use common pastures and herds that use common pastures. Association of PI animal prevalence with herd size was checked using Fischer exact test. Odds ratios regarding the association of antibody prevalence with use of common pastures was calculated using logistic regression. *P* value of < 0.05 was considered significant.

## Results

### Antibody prevalence

Apparent prevalence of specific antibodies in cattle was 59.57% (588/987); the true antibody prevalence was 61.61%. At the herd level, the estimated antibody prevalence was 100 %. The results of prevalence of specific BVD antibodies with regard to age category, herd size and grazing are summarized in Table 1. The observed difference of antibody prevalence between age categories was significant ( $P < 0.001$ ) with the highest prevalence in cows. Antibody prevalence between herds of different sizes was significant ( $P < 0.0001$ ) and increased proportionally with herd size. Furthermore, the prevalence of BVD antibodies was significantly different in herds that use common pastures compared to herds that do not ( $P < 0.001$ ). The frequency of BVD antibodies in herds that used common pasture was more than  $7 \times$  higher compared to herds that do not use common pastures (OR = 7.2; 95%CI 3.6-14.6).

Table 1. Seroprevalence of specific bovine viral diarrhoea virus antibodies in cattle with regard to category, herd size and grazing.

		n	Positive (%)
Categories	heifers	202	71 (35.2)
	cows	653	446 (68.3)
	calves	132	71 (53.8)
Herd size	< 20	339	133 (39.2)
	20–50	236	121 (51.3)
	50–100	294	222 (75.5)
	> 100	118	112 (94.9)
Grazing	without common pastures	896	506 (56.5)
	on common pastures	91	82 (90.1)

### Persistently infected cattle prevalence

Ear notch tissue samples were obtained from 2 284 cattle of 35 herds. Persistent infection was detected in 12 (0.53%) animals (5 cows, 2 pregnant heifers, 5 heifers) in 7 herds (Table 2). At the herd level, the prevalence was 20%. Prevalence of PI cattle was not associated with herd size ( $P < 0.9$ ).

### Management data

In all herds with up to 50 animals (except for one), cattle of all categories were placed together in one stable. In that one exception, herd heifers were placed in a separate stable before they became pregnant. In the herd where heifers were placed in a separate stable, persistently infected cattle was detected. In herds with 51 and more animals, heifers were placed separately from other categories before they became pregnant.

Table 2. Seroprevalence of persistently infected cattle with regard to category and herd size.

		n	Positive (%)
Categories	heifers	375	7 (1.9)
	cows	1674	5 (0.3)
	calves	235	0 (0)
Herd size	< 20	210	0 (0)
	20–50	226	1 (0.4)
	50–100	618	5 (0.8)
	> 100	1220	6 (0.5)
Total		2274	12 (0.5)

### Discussion

Until this study the impact of BVD in Croatia has never been investigated at a national level. According to this study, prevalence of BVDV antibodies in dairy herds was 61.6%. These data are in accordance with the data for the prevalence of 60–80% in most European countries (Lindberg et al. 2006). The highest prevalence was recorded in cows, followed by calves and heifers. Cows stay longer in herds compared to calves and heifers; therefore there bigger probability for them to become infected. Higher antibody prevalence in calves compared to heifers can be explained with colostrum antibodies. The lowest prevalence was recorded in heifers. This can be explained by the fact that in most cases on big farms heifers were placed separately until they became pregnant.

Although the prevalence of BVD antibodies at national level in Croatia is not extremely high, it is important that the estimated prevalence among herds with 100 and more cattle is 94.9%. Also, according to this study antibody prevalence is connected with the herd size. Large herds with 100 and more animals are the main source of milk production in Croatia and obviously the impact of BVD on those herds is enormous.

The prevalence of specific antibodies in herds with up to 50 animals that use common pastures was almost similar to herds with 100 and more animals that do not use common pastures. This result supports the finding that common pastures are a risk factor in BVD epidemiology (Valle et al. 1999).

On herd level, the estimated prevalence of antibodies was 100%. That means that each of the tested herds had at least one positive animal. However, in some herds the prevalence was low. The lowest antibody prevalence was observed among herds with 20 or less animals. This observation can explain previous computer modelling estimations that infection is contained earlier in smaller herds compared to larger herds (Smith et al. 2010).

The prevalence of PI animals and PI herds was 0.53% and 20%, respectively. These findings were in accordance with similar data from other countries (Rüfenacht et al. 2000).

The prevalence of PI herds was slightly below of the prevalence found in other European countries (Houe and Meyling 1991; Frey et al. 1996; Schreiber et al. 1999). These findings can be explained by the large number of herds with 20 or less animals in Croatia. Although the connection between frequency of virus prevalence and herd size in our study was not significant, 11 PI cattle of the 12 detected was found in six herds with more than 50 animals per herd. One of the reasons for that can be in different management techniques on small farms compared to large farms. Female calves on big farms were separated from the herds early after birth. Calves stay separately until they reach sexual maturity. After artificial insemination heifers are placed back into the

herd. For these reasons, heifers are seronegative. When heifers come infected with the virus during the second trimester of pregnancy, the virus can infect the foetus and cause persistent infection.

In each of the PI herds, PI cows or PI pregnant heifers were confirmed but not PI calves. Those findings are in conflict with the general presumption that PI animals commonly do not live long enough to reach reproductive maturity (Duffel and Harkness 1985). The reason that persistent infection has not been detected in calves can be just coincidence or such calves had been excluded from the herd because of growth retardation.

All positive animals were detected in the central, northern and eastern parts of Croatia. In those regions, the density of cattle population is high and these data support previous findings (Lindberg 2006).

In conclusion, BVD is present in Croatia in endemic form with seroprevalence of 61.61% and with PI animals prevalence of 0.53%. The finding of almost 95% antibody positive cattle in herds with 100 animals and more implies a major impact of BVD on dairy herds and milk production in Croatia. According to this study the main risk factor for the maintenance of the virus within herds is keeping heifers separately from the rest of the herd before they become pregnant. The obtained results suggest that changes in management may lead to the break of the chain of infection, and potentially to elimination of PI animals. Such changes in management can positively influence the results of control and eradication programs at national or even regional level.

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