



Original Contribution

SEROPREVALENCE AND RISK FACTORS OF BOVINE RESPIRATORY SYNCYTIAL VIRUS IN DAIRY AND BEEF CATTLE HERDS IN REPUBLIC OF BULGARIA

P. Marutsov*, B. Boneva-Marutsova

Department of Veterinary Microbiology, Infectious and Parasitic Diseases,
Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

Bovine respiratory syncytial virus (BRSV) is one of the important causative agents of Bovine respiratory disease complex (BRDC) in cattle all over the world. The present study was carried out to determine the seroprevalence of BRSV in dairy and beef cattle farms in Bulgaria. A total of 418 serum samples from 19 farms were tested using a commercial ELISA antibody test kit. The average rate of seroprevalence was 78.9 % indicating a high level of exposure to the virus.

In dairy and beef farms the seropositivity of BRSV was found to be 81.8%, and 72.7%, respectively. Evaluation of some risk factors reveals that the BRSV antibody prevalence was related to age, herd size, import of animals and season. This study demonstrates that infection is one of the common reasons of respiratory diseases in cattle which may have a significant influence on health and performance.

Key words: Bovine respiratory syncytial virus, seroprevalence, dairy cattle, beef cattle

INTRODUCTION

Bovine respiratory syncytial virus (BRSV) is one of the major viruses included in Bovine respiratory disease complex (BRDC) etiology (1). The virus is capable of infecting cattle on dairy and beef farms, resulting in clinical or subclinical infection. The initial infection by BRSV suppresses the immune system and facilitates secondary bacterial infection of the lower respiratory tract (2). BRSV is an RNA virus with a non-segmented, single-stranded, negative-sense 15.2 kb genome that belongs to the genus *Pneumovirus*. The virus is spread in the herd through airborne transmission and contact (direct or indirect) between infected animals. The horizontal transmission in combination with subclinical re-infection, is considered the main mechanism for circulation of BRSV within a population (3). The epidemiological studies reported that the prevalence of BRSV in cattle ranged from 28%

to 70%, depending on animal age and environmental conditions (4, 5). The disease occurs in most countries worldwide and affects cattle of all ages, with younger animals being at the greatest risk of clinical disease (6, 7). In disease outbreaks, morbidity is high (60-80%) and mortality can reach up to 20%, particularly in young calves (8, 9). Serosurveys, or studies capturing data on post-exposure antibodies to BRSV, help us understand true rates of infection at animal and farm level prevalence (10). In unvaccinated populations, seroprevalence generally increases with age but the effects of other factors such as herd size are inconsistent (11). Up-to-date information about the prevalence of BRSV in Bulgaria is limited. Earlier studies were made in the 1980s in 50 farms with a history of BRD, and the established seroprevalence by using the blocking ELISA test was 47.3% (12). Over the last 20 years, a change in the type, size, productivity and management of farms has occurred in our country. Thousands of cattle of the Holstein-Friesian genotype and various beef breeds were purchased. This probably affects disease rates, as in some cases disease outbreaks

*Correspondence to: *Plamen Marutsov, Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria*

were detected soon after the commingling of purchased and local cattle.

The aim, of this study was to estimate the seroprevalence of BRSV in beef and dairy cattle herds in Bulgaria and to assess the role of some risk factors associated with the disease.

MATERIALS AND METHODS

Study design and sample size

The study was conducted between March 2021 and April 2022. A total of 12 dairy and 7 beef farms from northern and southern Bulgaria were included in the survey. Cattle of different ages and breeds with no vaccination history against BRSV were tested in the study.

The sample size was calculated according to the method describe by Naing and colleagues (13) with an expected disease prevalence of 50%, confidence level of 95%, and standard error rate of 5%, using the following equation: $n = Z^2 \times P(1 - P)/d^2$. Where n = sample size, Z = Z statistic for a level of confidence (for the level of confidence of 95%, which is conventional, Z value is 1.96), P = expected prevalence or proportion (in the proportion of one; if 50%, $P = 0.5$), and d = precision (in proportion of one; if 5%, $d = 0.05$).

According to the calculation, a minimum of 384 samples were required to conduct this study. More than the equation required samples were obtained and included in the study. Calves less than 150 days of age were not included to avoid interference from maternal-derived antibodies.

Sampling and shipment

A total of 421 blood samples were collected. Blood was collected into sterile vacutainer tubes (VACUTEST KIMA, Italy) by venipuncture of the coccygeal or jugular vein. Samples remained at room temperature for 45 min for coagulation and after being transported to the laboratory by using a cool box and reusable dry gel packs. Samples were centrifuged at 3,000 rpm for 10 min to obtain the sera. The obtained sera were aliquoted in 1.5 mL identified micro tubes that were stored at -20 °C until analyses.

Laboratory analysis

The diluted samples (10 μ l serum in 190 μ l dilution buffer) were tested for the presence of circulating antibodies to the bovine respiratory syncytial virus by using a commercially available IDEXX RSV IgG Ab ELISA test kit (IDEXX Laboratories, Inc., *Manufacturer* IDEXX Montpellier SAS, France). The testing

procedure and interpretation of the results were done according to the manufacturer's recommendations. Samples showing S/P values $<20\%$ were considered negative, while those showing $S/P \% \geq 20$ were considered positive. Inconclusive samples were not retested, and were not included in further analysis. Measurement and reading of the optical densities were made at 450nm wavelength by using LED-based Microplate Reader Ledetect 96.

Risk factors

Epidemiological information about animal origin and geolocation, age structure, type, sex, number of cattle in the herd, import of animals and season, was collected by using a pre-designed questionnaire. It was administered to the owner or farm manager with the purpose of identifying risk factors related to the epidemiology of BRSV. The design was according to data of risk factors described in the literature (14, 15). The variables explored were: age group (≤ 12 months old, > 12 months old), type (dairy, beef), sex (female, male), herd size (≤ 200 , > 200 animals), purchase of animals (yes, no), season (October – March, April – September) and bovine viral diarrhoea virus - BVDV (positive, negative). Due to the widespread prevalence of BVDV, the clarification of its role as a risk factor leading to impaired immune function and the subsequent effect on the prevalence of BVDV was also assessed. Based on farm seroprevalence data for BVDV, dairy farms were divided into positive and negative (unpublished data). For BVDV dairy farms were determined as BVDV-free, when the results of all tested samples were negative. In each dairy farm, sampling was based on the stratification by age into five classes: <6 months old calves, 6–12 months old calves, pregnant heifers, uniparous, pluriparous (16).

STATISTICAL ANALYSIS

The two-sided Chi-square test was used to assess the difference in BRSV prevalence and various risk factors in the different cattle groups. Cramér's V is an effect size measurement for the chi-square test of independence, it measures how strongly two categorical fields are associated. The rate of relative ratio (RR) between BRSV risk factors was calculated at 95% significance. Statistical analyses were performed using GraphPad software (Graphpad Software Inc., San Diego, CA) with statistical significance set at $P < 0.05$.

Epidemiological analysis of risk difference (RD), attributable risk percent (AR%) and percent relative effect (%RE) with confidence intervals were calculated by using OpenEpi Version 3.01 (17).

RESULTS

As a result of ELISA testing, 3 samples were determined as inconclusive, the remaining 418 animals were included in the analysis. In 11 of 12 dairy farms (91.66%) and all of the beef farms included in the survey were detected antibodies to BRSV. The results of the ELISA antibody test revealed that the overall seroprevalence of BRSV was 78.9 %, (330 /418). The remaining 88 (21.05%) animals were determined as seronegative. The chi-square test showed significant associations between the presence of BRSV antibodies to all of the investigated risk factors ($P < 0.05$), except sex. Potential risk factors associated with higher BRSV seroprevalence are presented in **Table 1**. The highest prevalence 91.4% was observed in cattle that were aged > 12 months ($RR = 1.274$) compared with youngstock aged between 6 and

12 months. Small but significant differences in seroreactivity were found between dairy and beef farms, 81.8% and 72.7%, respectively (Cramer's $V = 0.22$, $p = 0.0502$). There was no significant difference between the seroprevalence of the BRSV infection in male and female animals in both dairy and beef farms. BRSV seropositivity for large herds (> 200 animals) was 90,5 %, ($RR = 2.52$), compared to those from small herds. In dairy farms (10/12) with a history of purchasing dairy heifers compared to dairy farms that don't import heifers, seropositivity was 91%, ($RR = 2.5102$) and 36,1%, respectively. All beef farms had a history of purchasing animals, therefore no risk assessment was performed. Samples collected from October to March displayed higher antibody prevalence ($RR = 1.17$, 95%, CI 1.05-1.30) compared to those collected during the rest of the year. Seropositive values of BVDV tested samples showed unequivocal association with BRSV seroprevalence ($RR = 1.22$, 95%, CI 1.05 - 1.42) in the chi-square test (**Table 2**).

Table 1. Relative risk factors associated with the prevalence of bovine respiratory syncytial virus in cattle.

Factors	Number of tested	Number of positive (%)	Cumulative incidence	RR	Confidence Limits	Significance level p-value
Age						
> 12 months	152	139 (91.4)	0.914	1.27	1.16-1.39	<0.0001
6 - 12 months	266	191 (71.8)	0.718			
Type						
Dairy	286	234 (81.8)	0.818	1.12	0.99-1.26	0.0502
Beef	132	96 (72.7)	0.727			
Sex						
Dairy female	243	202 (83.1)	0.831	1.12	0.92-1.34	0.2506
Dairy male	43	32 (74.4)	0.744			
Beef female	97	72 (74.2)	0.742	1.08	0.84-1.39	0.6714
Beef male	35	24 (68.5)	0.685			
Herd size						
Small ≤ 200	89	32 (35.9)	0.359			
Large ≥ 200	329	298 (90.5)	0.905	2.51	1.90- 3.33	< 0.0001
Import of dairy heifers						
yes	239	211 (88.3)	0.883	1.26	1.038 - 1.523	0.00147
no	47	33 (70.2)	0.702			
Season						
October – March	249	209 (83.9%)	0.839	1.172	1.05-1.30	0.0044
April – September	169	121 (71.5%)	0.715			

Table 2. Risk-based estimates of BVDV serostatus associated with BRSV seroprevalence in 286 animals from 12 dairy herds in Bulgaria.

	Number of tested animals	Number of positive (%)	Cumulative incidence CI	RR Confidence Limits (CL)	RD (%) (CL)	AR% (CL)	RE%
BVDV							
Pos	202	173 (85,6)	0.856	1.22 1.05 - 1.42	15.4 4.5 - 26.3	17.99 4.696 - 29.43	22
Neg	84	52 (70,2)	0.702				

$\chi^2=19.93$, p-value < 0.00001, Cramer's V 0,486

DISCUSSION

The overall seroprevalence of 78.9 %; obtained in this work does not differ greatly from reports from other researchers in Sweden, Iraq, Estonia, USA, Turkey (9; 18 - 22). The high levels of seroprevalence can be explained with the contagious nature of the disease. However various risk factors related to farm management, control and prevention strategic measures can affect the frequency of disease distribution. The highest BRSV seroprevalence was detected in older animals above 12 months compared to those from the younger age group, which is consistent with the findings of the previous studies (20, 21, 23). High disease seroreactivity in older herd mates is a result of possible re-infections with the virus during the course of their life. The results of this study demonstrated also that BRSV infection was common in both dairy and beef cattle at 81.8% and 72.7%, respectively (p=0.0502). In this case, Cramer's V coefficient (Cramer's V = 0.22) indicates that the effect of association was moderate. An earlier study done in Poland shows that the obtained seropositivity results in both dairy and beef calves (6 to 12 months old) were similar 35.5% and 48% (24). A study from Turkey showed that BRSV is more prevalent among dairy herds compared with beef herds (9). The authors explained their results with inadequate biosecurity in dairy herds. We can assume that the differences between dairy and beef herds are mostly due to farm management, confined rearing, poor air condition, and increased stocking density in dairy farms which facilitate the overall virus transmission. Other studies also explained the high seroprevalence of BRSV in dairy herds with intensive animal farming, increased herd traffic by herd employees for milk delivery or artificial insemination, and stress induced by practices to produce high milk yields (25, 26). The seroprevalence was very similar in female and male animals, which was also observed previously (27, 28) This finding is not unusual as animals of both sexes share the same farm

environment. In recent years, for economic reasons more and more dairy farms in Bulgaria leave their male calves for fattening, which increases contacts between animals and favors the transmission of pathogens. The findings of a higher prevalence rate in large farms (>200 animals) compared to small ones are in agreement with those of the other studies (20, 23, 29, 30). This can be explained based on the fact that overcrowding and frequent inter-animal contacts, will affect positively pathogen loads and transmission. In this study we also showed that the prevalence was significantly higher in dairy farms with the import of animals, the same was demonstrated in studies by other authors (31). Some researchers reported no association between the import of animals and BRSV seropositivity (9, 32). In infectious diseases, incidence usually increases after the introduction of infected or susceptible animals, especially when animals from multiple sources are commingled. The disease caused by bovine pestivirus (BVDV) is very common, in countries without eradication programs the herd level prevalence ranges from 60% to 85% (33). BVDV infection results in impairment of immune function and consequent decrease in resistance, which could explain the positive association with BRSV serostatus. The synergistic effects of BVDV with other respiratory pathogens included in BRD have been observed earlier (32).

CONCLUSION

BRSV seroprevalence in Bulgaria is higher, but similar to that in other parts of the world. The presence of antibodies indicates previous exposure to the virus but also it is evidence of virus circulation in the population. The established distribution of BRSV in dairy and beef farms and the incrimination of the risk factors are important for refining prevention and control measures in order to manage BRD. Based on this, it is necessary to develop operational strategies for external and internal biosecurity and adopt regular vaccinations to

improve health and productivity in cattle herds. This will reduce the infectious pressure, economic losses and indirectly lead to a reduction in the use of antimicrobials for treatment and metaphylaxis.

REFERENCES

1. Buczinski, S., & Pardon, B., Bovine respiratory disease diagnosis: What progress has been made in clinical diagnosis? *Veterinary Clinics: Food Animal Practice*, 36(2), 399-423, 2020.
2. Sudaryatma, P. E., Nakamura, K., Mekata, H., Sekiguchi, S., Kubo, M., Kobayashi, I., ... & Okabayashi, T., Bovine respiratory syncytial virus infection enhances *Pasteurella multocida* adherence on respiratory epithelial cells. *Veterinary microbiology*, 220, 33-38, 2018.
3. Makoschey, B., Berge, A.C., Review on bovine respiratory syncytial virus and bovine parainfluenza – usual suspects in bovine respiratory disease – a narrative review. *BMC Vet Res* 17, 261, 2021. <https://doi.org/10.1186/s12917-021-02935-5>.
4. Ellis J, West K, Knobby C, Leard T, Gallo G, Colon J, Fitzgerald N., Efficacy of an inactivated respiratory syncytial virus vaccine in calves. *J. Am. Vet. Med. Assoc.*, 218(12):1973–1980, 2001.
5. Sarmiento-Silva R.E, Nakamura-Lopez Y, Vaughan G. Epidemiology, molecular epidemiology and evolution of bovine respiratory syncytial virus. *Viruses.*, 4(12):3452–3467, 2012.
6. Stokstad M, Klem TB, Myrmel M, Oma VS, Toftaker I, Østerås O and Nødtvedt A., Using Biosecurity Measures to Combat Respiratory Disease in Cattle: The Norwegian Control Program for Bovine Respiratory Syncytial Virus and Bovine Coronavirus. *Front. Vet. Sci.*, 7:167, 2020. doi: 10.3389/fvets.2020.00167.
7. Morel-Journel, T., Assié, S., Vergu, E., Mercier, J. B., Bonnet-Beaugrand, F., & Ezanno, P., Minimizing the number of origins in batches of weaned calves to reduce their risks of developing bovine respiratory diseases. *Veterinary Research*, 52, 1-12., 2021.
8. Valarcher, J.F., and Taylor, G., Bovine respiratory syncytial virus infection, *Veterinary Research*, 38, 153-180, 2007.
9. İnce, Ö. B., Şevik, M., Özgür, E. G., & Sait, A. Risk factors and genetic characterization of bovine respiratory syncytial virus in the inner Aegean Region, Turkey. *Tropical Animal Health and Production*, 54(1), 4, 2022.
10. Hägglund, S., Näslund, K., Svensson, A., Lefverman, C., Enül, H., Pascal, L., ... & Valarcher, J. F., Longitudinal study of the immune response and memory following natural bovine respiratory syncytial virus infections in cattle of different age. *Plos one*, 17(9), e0274332, 2022.
11. Solís-Calderón, J. J., Segura-Correa, J. C., Aguilar-Romero, F. & Segura-Correa, V. M., Detection of antibodies and risk factors for infection with bovine respiratory syncytial virus and parainfluenza virus-3 in beef cattle of Yucatan, Mexico. *Preventive Veterinary Medicine*, 82, 102-110, 2007.
12. Bostandjieva R., Cultivation and diagnosis of Bovine respiratory syncytial virus. PhD Thesis. National Diagnostic Research Institute Of Veterinary Medicine "Prof. Dr. G. PAVLOV", Sofia, Bulgaria, 1987.
13. Naing, L., Winn, T. B. N. R., & Rusli, B. N., Practical issues in calculating the sample size for prevalence studies. *Archives of orofacial Sciences*, 1, 9-14, 2006.
14. Luzzago C, Bronzo V, Salvetti S, Frigerio M, Ferrari N. Bovine respiratory syncytial virus seroprevalence and risk factors in endemic dairy cattle herds. *Vet Res Commun.* 34:19–24, 2010.
15. Ohlson A, Heuer C, Lockhart C, Travén M, Emanuelson U, Alenius S. Risk factors for seropositivity to bovine coronavirus and bovine respiratory syncytial virus in dairy herds. *Vet Rec.*, 167:201–6, 2010.
16. Luzzago, C., Piccinini, R., Zeponi, A., & Zeconi, A., Study on prevalence of bovine viral diarrhoea virus (BVDV) antibodies in 29 Italian dairy herds with reproductive problems. *Veterinary Microbiology*, 64(2-3), 247-252, 1999.
17. González-Ramírez, A. R., and F. Rivas-Ruiz. "Measures of frequency, magnitude of association and impact in epidemiology." *Allergologia et immunopathologia* 38, 147-152, 2010.
18. Bidokhti, M. R., Trávén, M., Fall, N., Emanuelson, U., & Alenius, S., Reduced likelihood of bovine coronavirus and bovine respiratory syncytial virus infection on organic compared to conventional dairy farms. *The Veterinary Journal*, 182(3), 436-440, 2009.
19. Beaudéau F., A. Ohlson, U. Emanuelson. Associations between bovine coronavirus and bovine respiratory syncytial virus infections and animal performance in

- Swedish dairy herds. *J. Dairy Sci.*, 93, pp. 1523-1533, 2010. 10.3168/jds.2009-2511
20. Hussain KJ, Al-Farwachi MI, Hassan SD. Seroprevalence and risk factors of bovine respiratory syncytial virus in cattle in the Nineveh Governorate, *Iraq. Vet World*. Nov;12(11):1862-1865, 2019.
 21. Mõtus, K., Rilanto, T., Viidu, D.A., Orro, T. and Viltrop, A., Seroprevalence of selected endemic infectious diseases in large-scale Estonian dairy herds and their associations with cow longevity and culling rates. *Preventive Veterinary Medicine*, 192, p.105389. 2021.
 22. Martinez, D. A., Newcomer, B., Passler, T., & Chamorro, M. F., Efficacy of bovine respiratory syncytial virus vaccines to reduce morbidity and mortality in calves within experimental infection models: a systematic review and meta-analysis. *Frontiers in Veterinary Science*, 9, 906636, 2022.
 23. León JCP, Diaz W, Vasquez MC, Tobón JC, Sánchez A, Ortiz D. Seroprevalence and risk factor associated with respiratory viral pathogens in dual-purpose cattle of Aguachica, Rio de Oro, and La Gloria municipalities in Cesar department, Colombia. *Vet World*. Jul;12(7):951-958, 2019.
 24. Urban-Chmiel, R., Wernicki, A., Puchalski, A., Dec, M., Stęgierska, D., Grooms, D. L., & Barbu, N. I., Detection of bovine respiratory syncytial virus infections in young dairy and beef cattle in Poland. *Veterinary Quarterly*, 35(1), 33-36, 2015.
 25. Norström, M., Skjerve, E., Jarp J., Risk factors for epidemic respiratory disease in Norwegian cattle herds, *Preventive Veterinary Medicine*, 44, 87-96, 2000.
 26. Dahl, G.E., Tao, S., Laporta, J., Heat stress impacts immune status in cows across the life cycle, *Frontiers in Veterinary Science*, 7, 116, 2020.
 27. Hägglund S, Hu K.F, Larsen L.E, Hakhverdyan M, Valarcher J.F. Bovine respiratory syncytial virus ISCOMs-protection in the presence of maternal antibodies. *Vaccine*. 23(5):646–655, 2004.
 28. Assié, S., Bareille, N., Beaudeau, F., & Seegers, H., Management-and housing-related risk factors of respiratory disorders in non-weaned French Charolais calves. *Preventive veterinary medicine*, 91(2-4), 218-225, 2009.
 29. Saa L.R, Perea A, Jara D.V, Arenas A.J, Garcia-Bocanegra I, Borge C, Carbonero A. Prevalence and risk factors for bovine respiratory syncytial virus (BRSV) infection in non-vaccinated dairy and dual-purpose cattle herds in Ecuador. *Trop. Anim. Health Prod.*, 44(7):1423–1427, 2012.
 30. Ferella A, Aguirreburualde M, Marginedac C, Aznarb N, Sammarrucoa A, Dus Santosa M, Mozgovoj M. Bovine respiratory syncytial virus seroprevalence and risk factors in feedlot cattle from Córdoba and Santa Fe, Argentina. *Rev. Argent. Microbiol.*, 50(3):275–279, 2018.
 31. Elvander, M., Severe respiratory disease in dairy cows caused by infection with bovine respiratory syncytial virus, *Veterinary Record*, 138,101-105, 1996.
 32. Hoppe, I. B. A. L., Medeiros, A. S. R. D., Arns, C. W., & Samara, S. I., Bovine respiratory syncytial virus seroprevalence and risk factors in non-vaccinated dairy cattle herds in Brazil. *BMC veterinary research*, 14, 1-6, 2018.
 33. Houe, Hans. "Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections." *Veterinary microbiology* 64, no. 2-3 (1999): 89-107.