

Detection of antibodies against bovine respiratory syncytial virus (BRSV) in dairy cattle with different prevalences of bovine herpesvirus type 1 (BoHV-1) in São Paulo State, Brazil

Detecção de anticorpos contra o vírus respiratório sincicial bovino (BRSV) em rebanhos leiteiros com diferentes prevalências de herpesvirus bovino tipo 1 (BoHV-1) no Estado de São Paulo, Brasil

Ingrid Bortolin Affonso¹; Sandra Possebon Gatti²; Bruna Alexandrino³;
Mônica Costa Oliveira⁴; Andréa Souza Ramos de Medeiros⁵;
Maria da Glória Buzinaro⁶; Samir Issa Samara^{7*}

Abstract

Serum samples from three dairy farms classified as high (1, 78.6%), medium (2, 40.0%) and low (3, 1.6%) prevalence for bovine herpesvirus type 1 (BoHV-1) from São Paulo State, were collected to analyze the prevalence to the bovine respiratory syncytial virus (BRSV) by virus neutralization test. The BRSV observed prevalences for farms 1, 2, and 3 were 45.61%, 84.42%, and 54.09%, respectively. Farm 2 showed higher prevalence to BRSV ($P < 0.0001$) than farms 1 and 3, which were equivalent ($P = 0.2291$). Two risk factors, herd size and milder climate conditions, were associated to BRSV. The prevalence of BRSV in farm 1 was statistically lower than BoHV-1 prevalence ($P < 0.0001$). On the other side, farms 2 and 3 have prevalences of BRSV higher than of BoHV-1 ($P < 0.0001$ in both comparisons). We may conclude, at least for the studied farms, that BRSV and BoHV-1 did not interact and the differences observed between BRSV and BoHV-1 prevalences into each herd did not have epidemiological importance.

Key words: Bovine respiratory syncytial virus, bovine herpesvirus type 1, prevalence, virus neutralization test, Brazil

Resumo

Amostras de soro de três fazendas leiteiras do Estado de São Paulo, classificadas como de alta (1, 78,6%), média (2, 40,0%) e baixa (3, 1,6%) prevalência de herpesvirus bovino tipo 1 (BoHV-1) foram coletadas para analisar a prevalência do vírus respiratório sincicial bovino (BRSV) pelo teste

¹ Doutoranda do Programa de Pós-Graduação em Medicina Veterinária, Faculdade de Ciências Agrárias e Veterinárias, Campus de Jaboticabal, Universidade Estadual Paulista. E-mail: ibaffonso@gmail.com

² Prof^a. Dr^a., Colégio Técnico Agrícola “José Bonifácio”, Faculdade de Ciências Agrárias e Veterinárias, Campus de Jaboticabal, Universidade Estadual Paulista. E-mail: sandra.gatti@cta.unesp.br

³ Doutoranda do Programa de Pós-Graduação em Medicina Veterinária, Faculdade de Ciências Agrárias e Veterinárias, Campus de Jaboticabal, Universidade Estadual Paulista. E-mail: bralexandrino@yahoo.com.br

⁴ Doutoranda do Programa de Pós-Graduação em Medicina Veterinária, Faculdade de Ciências Agrárias e Veterinárias, Campus de Jaboticabal, Universidade Estadual Paulista. E-mail: vetmonica@yahoo.com.br

⁵ Assistente de suporte acadêmico I, Departamento de Medicina Veterinária Preventiva e Reprodução Animal, Faculdade de Ciências Agrárias e Veterinárias, Campus de Jaboticabal, Universidade Estadual Paulista. E-mail: andrea@fcav.unesp.br

⁶ Prof^a. Assistente Doutora, Departamento de Medicina Veterinária Preventiva e Reprodução Animal, Faculdade de Ciências Agrárias e Veterinárias, Campus de Jaboticabal, Universidade Estadual Paulista. E-mail: glorinha@fcav.unesp.br

⁷ Prof. Titular, Departamento de Medicina Veterinária Preventiva e Reprodução Animal, Faculdade de Ciências Agrárias e Veterinárias, Campus de Jaboticabal, Universidade Estadual Paulista. E-mail: samara@fcav.unesp.br

* Autor para correspondência

de virusneutralização. As prevalências de BRSV observadas nas fazendas 1, 2 e 3 foram 45,61%, 84,42% e 54,09%, respectivamente. A fazenda 2 apresentou maior prevalência de BRSV ($P < 0,0001$) do que fazendas 1 e 3, que foram equivalentes ($P = 0,2291$). Dois fatores de risco, tamanho do rebanho e condições climáticas, foram associados ao BRSV. A prevalência de BRSV na fazenda 1 foi estatisticamente inferior à prevalência de BoHV-1 ($P < 0,0001$). Por outro lado, as fazendas 2 e 3 possuíram prevalências de BRSV superiores às de BoHV-1 ($P < 0,0001$ em ambas as comparações). Podemos concluir, pelo menos para as fazendas estudadas, que o BRSV e BoHV-1 não interagiram, e que as diferenças observadas entre as prevalências de BRSV e BoHV-1 em cada rebanho não tiveram importância epidemiológica.

Palavras-chave: Vírus respiratório sincicial bovino, herpesvirus bovino tipo 1, prevalência, teste de virusneutralização, Brasil

Introduction

The bovine respiratory syncytial virus (BRSV), member of the *Paramyxoviridae* family, genus *Pneumovirus* (MURPHY et al., 1995), is one of the most important causes of lower respiratory tract infections in calves (LARSEN, 2000). This virus, along with other viruses such as bovine herpesvirus type-1 (BoHV-1), bovine parainfluenza type 3 (PI-3), bovine viral diarrhea virus (BVDV), bovine adenovirus serotype 1 (BAV-1), and serotype 3 (BAV-3), bacteria as *Mannheimia haemolytica*, *Pasteurella multocida*, *Haemophilus somnus*, and *Mycoplasma* spp constitute the bovine respiratory complex (DURHAM et al., 1991).

Due to the intensive livestock, this virus has become a problem in several countries. According to Hoerlein (1973), factors as stress, management, and environmental conditions are related to the bovine respiratory complex. Since the BRSV discovery in Switzerland by Paccaud and Jacquier (1970), it has been described worldwide (BAKER; AMES, 1993) usually concomitantly to other viruses (DURHAM; HASSARD, 1990; OBANDO et al., 1999; FULTON et al., 2000; SOLÍS-CALDERÓN et al., 2007; YESILBAG; GÜNGÖR, 2008).

In Brazil, BRSV was first diagnosed in calves from Rio Grande do Sul State by Gonçalves et al. (1993) and since then have been sparsely reported (CAMPALANS; ARNS, 1997; PEIXOTO et al., 2000; ALMEIDA et al., 2006).

In contrast, the epidemiology of the BoHV-1, a virus frequently related to respiratory and

reproductive diseases which leads to important economic losses (VAN DONKERSGOED; BABIUK, 1991; HAGE; SCHUKKEN; BARKEMA, 1996), is well known in Brazil. This pathogen has been diagnosed in different Brazilian regions, with prevalence rates ranging from 18% to 90% (LOVATO et al., 1995; RICHTZENHAIN et al., 1999; OKUDA et al., 2006).

Due to the lack of data on the prevalence of BRSV in Southeastern Brazil and on its relationship with other viruses such as BoHV-1, this study aims to determine the prevalence of this virus in three dairy farms in São Paulo State with different prevalence for the BoHV-1 and its association with this virus.

Materials and Methods

Study Area

This study was performed on three dairy farms from the municipalities of Viradouro (1), Altinópolis (2) and Jaboticabal (3), with herds of 140, 380 and 80 animals, respectively, all situated in São Paulo State, Southeastern Brazil. In a previous virus neutralization test epidemiological study (MOREIRA, 2004), prevalences of BoHV-1 in these farms were classified as high (farm 1, 78.6%), medium (farm 2, 40.0%) and low (farm 3, 1.6%), after Del Fava (2002). Farms 1 and 3 have similar elevation and climatic conditions above sea level (500m., Aw Köppen climate classification), while farm 2 is located at 900 m. above sea level,

with Cwa Köppen classification (CEPAGRI, 2009). Vaccination protocols for bovine respiratory complex diseases were not employed in these farms.

Sample determination

Samples size was determined after Astudillo (1979), with estimated BRSV prevalence of 80%, following data of Arns (1996). Therefore, the calculated representative samples for farms 1, 2, and 3 were 57, 77, and 44 animals. Sera from adult animals were collected, identified and stored at -20°C until testing.

Virus neutralization test

Serum samples were thawed, inactivated in water bath at 56°C for 30 minutes, and diluted in duplicates from 1:2 to 1:1024 in 96-well microplates, with $50\mu\text{L}$ of 200 TCID_{50} BRSV suspended in Eagle's minimum essential medium (DIFCO E-MEM®). The viral strain was previously titled after Reed and Muench (1938). Following incubation at 37°C in 5% CO_2 atmosphere for 1 h, $50\mu\text{L}$ of a suspension of Madin & Darbin bovine kidney (MDBK) cells in E-MEM and 10% bovine fetal serum solution were added to the wells, and the plates were re-incubated in similar conditions for 96 h. Each test included a back titration and cell culture control. Samples were positive when cytopathic effect was inhibited at 1:2 dilution and the further dilutions will be considered for future studies. Analytical procedures followed São Paulo Biological Institute recommendations.

Statistical analysis

Fischer's exact test was employed to compare the BRSV seroprevalences of the three farms. The same statistical procedure was used to compare the BRSV and BoHV-1 prevalences in each farm. Statistical analyses were performed by GraphPad® Prism v. 5.0 software. P was set in 0.05.

Results and Discussion

The observed BRSV prevalences for farms 1, 2, and 3 were 45.61%, 84.42%, and 54.09%, respectively. Fischer's exact test revealed difference of the prevalence of farm 2 in comparison to farms 1 and 3 ($P<0.0001$ and $P=0.0039$), which showed equivalent prevalences ($P=0.2291$). The prevalence of BRSV in farm 1 was lower than BoHV-1 prevalence ($P=0.0004$). On the other side, farms 2 and 3 have prevalences of BRSV higher than of BoHV-1 ($P<0.0001$ in both comparisons).

Several studies state that BRSV have crucial role in the development of respiratory disease in cattle herds, especially in dairy farms (KIMMAN et al., 1988; LARSEN, 2000). Seroepidemiological surveys for BRSV in different countries often reveal prevalences higher than 40% (DURHAM; HASSARD, 1990; OBANDO et al., 1999; SOLÍS-CALDERÓN et al., 2007). In Brazil, prevalences of this viral disease ranged from 68% to 91% in herds from Rio Grande do Sul state, southern Brazil (ARNS, 1996; CAMPALANS; ARNS, 1997). In São Paulo State there are reports of BRSV occurrence both in beef and dairy cattle, with or without respiratory signs, in prevalences up to 87% (ARNS, 1996). It is difficult to compare the prevalences obtained in this study with previous reports, as the herds are different and little is known on concomitant diseases association. However, even the lowest prevalence observed on the farms of this study was close to 50%, and so we may suggest that BRSV prevalence in Brazilian herds is often high, similar to those observed in other countries.

Despite the fact that the studied farms employed equivalent management procedures and sanitary protocols, BRSV prevalence on farm 2 was higher than in the other studied farms. If there were any interactions of BoHV-1 and BRSV, statistical differences would be expected between farms 1 and 3, as prevalences of BoHV-1 in these herds are strongly discrepant. Therefore, we could conclude that, at least in the studied farms, BRSV and BoHV-

1 did not interact, and the difference observed between BRSV and BoHV-1 prevalences into each herd did not have epidemiological importance.

Regarding this data, two risk factors may be associated to BRSV: herd size and climate conditions resultant from altitude. Previous studies showed high odds of seropositivity in animals from large herds (NORSTRÖM; SKJERVE; JARP, 2000; SOLÍS-CALDERÓN et al., 2007; YESILBAG; GÜNGÖR, 2008), as the virus is more stable in densely populated herds due to the increased contact between animals.

Outbreaks of this disease often occur as environmental temperature falls in winter (PIRIE et al., 1981; MAHIN; SHIMI, 1982), revealing a climatic influence on BRSV epidemiology. So, as farm 2 is located in a high altitude Cwa region, the mild temperatures may favor the pathogen maintenance and transmission into the herd.

Conclusions

High prevalences of BRSV were observed in the studied farms, ranging from 45.61% to 84.42%, and were similar to data from other studies in several countries. There was no interaction between BRSV and BoHV-1, but climate conditions and herd size may be linked to BRSV epidemiology, as the farm in which this viral disease was diagnosed in higher prevalence have milder temperatures than the other farms and also the larger herd.

Acknowledgements

The authors would like to thank to Dr. Edviges Maristela Pituco and Dr. Claudia Pestana Ribeiro from São Paulo Biological Institute for providing the BRSV strain, VNT protocols and for technical assistance, Dr. Estevam G. Lux Hoppe for assistance with manuscript redaction, and also to FAPESP (Process number 2008/56693-6) for financial support. Affonso also thanks to CNPq for graduate scholarship (process number 561765/2008-1).

References

- ALMEIDA, R. S.; DOMINGUES, H. G.; SPILKI, F. R.; LARSEN, L. E.; HAGGLUND, S.; BELAK, S.; ARNS, C. W. Circulation of bovine respiratory syncytial virus in Brazil. *Veterinary Record*, Londres, v. 158, n. 18, p. 632-634, 2006.
- ARNES, C. W. Vírus respiratório sincicial dos bovinos (BRSV): Situação no Brasil. In: SIMPÓSIO PFIZER SOBRE DOENÇAS INFECCIOSAS E VACINAS PARA BOVINOS, 1., 1996, Guarulhos. *Anais...Guarulhos: [s.n]*, 1996. p. 37-39.
- ASTUDILLO, V. M. *Encuestas por muestreo para estudios epidemiológicos en poblaciones animales*. Centro Panamericano de Fiebre Aftosa. Rio de Janeiro: Editora Panamericana, 1979. p. 18-24.
- BAKER, J. C.; AMES, T. R. The epidemiology of BRSV infection. *Veterinary Medicine*, Lenexa, v. 88, n. 9, p. 881-885, 1993.
- CAMPALANS, J.; ARNS, C. W. Serological evidence of bovine respiratory syncytial virus in Brazil. *Virus Reviews and Research*, Belo Horizonte, v. 2, n. 1/2, p. 50-56, 1997.
- CEPAGRI. *Centro de Pesquisas Meteorológicas e Climáticas Aplicadas à Agricultura*. Disponível em: <<http://www.cpa.unicamp.br>>. Acesso em: 7 set. 2009.
- DEL FAVA, C.; PITUCO, E. M.; D'ANGELINO, J. L. Herpesvirus bovino tipo 1 (HVB-1): revisão e situação atual no Brasil. *Revista de Educação Continuada CRMV-SP*, São Paulo, v. 5, n. 3, p. 300-312, 2002.
- DURHAM, P. J. K.; HASSARD, L. E. Prevalence of antibodies to infectious bovine rhinotracheitis, parainfluenza 3, bovine respiratory syncytial, and bovine viral diarrhoea viruses in cattle in Saskatchewan and Alberta. *Canadian Veterinary Journal*, Ottawa, v. 31, n. 12, p. 815-820, 1990.
- DURHAM, P. J. K.; HASSARD, L. E.; VAN DONKERSGOED, J. Serological studies of infectious bovine rhinotracheitis, parainfluenza 3, bovine viral diarrhoea, and bovine respiratory syncytial viruses in calves following entry to a bull test station. *Canadian Veterinary Journal*, Ottawa, v. 32, n. 7, p. 427-429, 1991.
- FULTON, R. W.; PURDY, C. W.; CONFER, A. W.; SALIKI, J. T.; LOAN, R. W.; BRIGGS, R. E.; BURGE, L. J. Bovine viral diarrhoea infection in feeder calves with respiratory disease: Interactions with *Pasteurella* spp., parainfluenza-3 virus and bovine respiratory syncytial virus. *Canadian Journal of Veterinary Research*, Ottawa, v. 64, n. 3, p. 151-159, 2000.

- GONÇALVES, I. P. D.; SIMANKE, A. T.; JOST, H. C.; HÖTZEL, I.; DAL SOGLIO, A.; MOOJEN, V. Detection of bovine respiratory syncytial virus in calves of Rio Grande do Sul, Brazil. *Ciência Rural*, Santa Maria, v. 23, n. 3, p. 389-390, 1993.
- HAGE, J. J.; SCHUKKEN, Y. H.; BARKEMA, H. W. Population dynamics of bovine herpes 1 infection in a dairy herd. *Veterinary Microbiology*, Amsterdam, v. 53, n. 1/2, p. 317-343, 1996.
- HOERLEIN, A. B. Preconditioning of beef cattle. *Journal of the American Veterinary Medical Association*, Schaumburg, v. 163, n. 7, p. 825-827, 1973.
- KIMMAN, T. G.; ZIMMER, G. M.; WESTENBRINK, F.; MARS, J.; VANLEEUEWEN, E. Epidemiological study of bovine respiratory syncytial virus-infections in calves: Influence of maternal antibodies on the outcome of disease. *Veterinary Record*, Londres, v. 123, v. 4, p. 104-109, 1988.
- LARSEN, L. E. Bovine respiratory syncytial virus (BRSV): a review. *Acta Veterinaria Scandinavica*, Copenhagen, v. 41, n. 1, p. 1-24, 2000.
- LOVATO, L. T.; WEIBLEN, R.; TOBIAS, F. L.; MORAES, M. P. Herpesvirus bovino tipo 1 (HVB-1): Inquérito soro-epidemiológico no rebanho leiteiro do Estado do Rio Grande do Sul, Brasil. *Ciência Rural*, Santa Maria, v. 25, n. 3, p. 425-430, 1995.
- MAHIN, L.; SHIMI, A. Weather and BRSV infection. *Veterinary Record*, Londres, v. 111, n. 4, p. 87, 1982.
- MOREIRA, S. P. G. *Avaliação do desenvolvimento ponderal de bezerras em plantéis leiteiros infectados pelo herpesvírus bovino tipo 1 (BHV-1)*. 2004. Tese (Doutorado em Medicina Veterinária Preventiva) – Faculdade de Ciências Agrárias e Veterinárias. Universidade Estadual Paulista, Jaboticabal.
- MURPHY, F. A.; FAUQUET, C. M.; BISHOP, D. H. L.; GHABRIAL, S. A.; JARVIS, A. W.; MARTELLI, G. P.; MAYO, M. A.; SUMMERS, M. D. Virus taxonomy. *Archives of Virology*, New York, p. 268-274, 1995. Suplemento 10.
- NORSTRÖM, M.; SKJERVE, E.; JARP, J. Risk factors for epidemic respiratory disease in Norwegian cattle herds. *Preventive Veterinary Medicine*, Amsterdam, v. 44, n. 1, p. 87-96, 2000.
- OBANDO, R. C.; HIDALGO, M.; MERZA, M.; MONTOYA, A.; KLINGEBORN, B.; MORENO, L. J. Seroprevalence to bovine virus diarrhoea virus and other viruses of the bovine respiratory complex in Venezuela (Apure State). *Preventive Veterinary Medicine*, Amsterdam, v. 41, n. 4, p. 271-278, 1999.
- OKUDA, L. H.; AGUIAR, D. M.; CAVALCANTE, G. T.; STEFANO, E.; DEL FAVA, C.; PITUCO, E. M.; LABRUNA, M. B.; CAMARGO, L. M. A.; GENNARI, S. M. Inquérito soro-epidemiológico do herpesvírus bovino tipo 1 (BoHV-1) no município de Monte Negro, Estado de Rondônia, Brasil. *O Biológico*, São Paulo, v. 68, n. 2, p. 103-105, 2006.
- PACCAUD, M. F.; JACQUIER, C. L. A respiratory syncytial virus of bovine origin. *Archiv fuer die Gesamte Virusforschung*, Vienna, v. 30, n. 4, p. 327-342, 1970.
- PEIXOTO, P. V.; MOTA, R. A.; BRITO, M. F.; CORBELLINI, L. G.; DRIEMEIER, D.; DE SOUZA, M. I. Infecção natural pelo vírus sincicial respiratório bovino (BRSV) no Estado de Alagoas. *Pesquisa Veterinária Brasileira*, Rio de Janeiro, v. 20, n. 4, p. 171-175, 2000.
- PIRIE, H. M.; PETRIE, L.; PRINGLE, C. R.; ALLEN, E. M.; KENNEDY, G. J. Acute fatal pneumonia in calves due to respiratory syncytial virus. *Veterinary Record*, Londres, v. 108, n. 19, p. 411-416, 1981.
- REED, L. J.; MUENCH, H. A simple method of estimating 50 per cent end point. *American Journal of Hygiene*, Baltimore, v. 27, n. 3, p. 493-497, 1938.
- RICHTZENHAIN, L. J.; BARBARINI, O.; UMEHARA, O.; DE GRACIA, A. S.; CORTEZ, A.; HEINEMANN, M. B.; FERREIRA, F. Rinotraqueíte Infecçiosa Bovina: Levantamento sorológico nos Estados de Minas Gerais, Mato Grosso do Sul, São Paulo, Rio de Janeiro, Paraná e Rio Grande do Sul. *Arquivos do Instituto Biológico*, São Paulo, v. 66, n. 1, p. 83-88, 1999.
- SOLÍS-CALDERÓN, J. J.; SEGURA-CORREA, J. C.; AGUILAR-ROMERO, F.; SEGURA-CORREA, V. M. Detection of antibodies and risk factors for infections with bovine respiratory syncytial virus and parainfluenza virus-3 in beef cattle of Yucatan, Mexico. *Preventive Veterinary Medicine*, Amsterdam, v. 82, n. 1/2, p. 102-110, 2007.
- VAN DONKERSGOED, J.; BABIUK, L. A. Diagnosing and managing the respiratory form of infectious bovine rhinotracheitis. *Veterinary Medicine*, Lenexa, v. 86, n. 1, p. 86-94, 1991.
- YESILBAG, K.; GÜNGÖR, B. Seroprevalence of bovine respiratory viruses in North-western Turkey. *Tropical Animal Health Production*, Edinburgh, v. 40, n. 4, p. 55-60, 2008.

