International Journal of Advanced Engineering Research and Science (IJAERS)
Peer-Reviewed Journal
ISSN: 2349-6495(P) | 2456-1908(O)
Vol-9, Issue-9; Sep, 2022
Journal Home Page Available: https://ijaers.com/
Article DOI: https://dx.doi.org/10.22161/ijaers.99.66

Seroprevalence of Cattle Respiratory Viral Pathongens in Paranatinga, Mato Grosso State, Brazil

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Received: 29 Aug 2022,
Received in revised form: 18 Sep 2022,
Accepted: 23 Sep 2022,
Available online: 30 Sep 2022

***Keywords*— Bovine Hespesvirus Type 1, Bovine Respiratory Diseases Complex, Bovine Viral Diarrhea Virus, Feedlot, Parainfluenza Type 3 Virus**

***Abstract*—** *The Bovine Respiratory Diseases Complex (BRD) is characterized by respiratory tract infection and may be origin by viral, bacterial or through the association of both. BRD has been identified as one of the most important causes of morbidity and mortality of intensive breeding, especially in young animals, causing serious economic losses with the use of drugs for treatment of animals and significant losses of weight, directly affecting the meat production. The objective of this study was to verify the presence, through the serological and / or antigenic detection, of etiological agents that cause BRD in blood and nasal swab samples, such as Bovine Hespesvirus Type 1 (BoHV-1), Bovine Viral Diarrhea Virus, Parainfluenza Type 3 virus (BPI-3). There were evaluated 100 bulls from four properties in the municipality of Paranatinga sent for termination in a feedlot located in the municipality of Ipiranga do Norte, MT. According to the results obtained, a high prevalence of the viral agents studied was observed, mainly the BPI-3 and BoHV-1 viruses. The results indicate the circulation of agents that cause diseases of importance in beef cattle, thus requiring greater vigilance over them in feedlots.*

I. INTRODUCTION

Bovine livestock has been playing a role of great economic importance within the national agribusiness. Brazil has the largest commercial herd in the world and the second largest effective herd in the world, over 220 million heads (Brasil 2022). Within the meat production chain, intensive farming through confinement is an excellent alternative for the producer in off-season periods, as it provides greater use of the rural property area and has a fast cycle.

According to the Instituto Matogrossense de Economia Agropecuária (IMEA 2022) in 2020, about 860,000 head of cattle were confined in the state, where the mid-north region of Mato Grosso stands out, participating in 13,6% of the total of confined animals, highlighting, thus, the importance of the activity for the region.

Despite the productive increase in beef cattle, the intensive confinement system presents some health problems. In the United States, studies reveal that the Bovine Respiratory Disease Complex (BRD) is a major problem for the meat production chain. Characterized as the main cause of morbidity and mortality in feedlots, it contributes to significant losses in weight and carcass quality in cattle, in addition to generating costs with medicines for its treatment (Griffin 2014).

BRD is an important disease mainly for intensive breeding and young animals. Its etiopathogenesis is complex and composed of several environmental factors that combined with viral and bacterial infectious agents

end up suppressing and overloading the animal's immune system. The multiple factors end up conditioning the animal to a stressful situation, among them we can highlight the inadequate management of the herd, food imbalance, nutritional failures, transport and veterinary interventions (Guzman and Taylor 2015, Earley et al. 2017).

The infectious agents of the disease can act alone or in association, or also in a primary or secondary way. Of the viral agents, the most important are Parainfluenza virus type 3 (BPI-3), Bovine Herpesvirus type 1 (BHV-1) and Bovine Viral Diarrhea Virus (BVD) (JARED et al. 2010, Earley et al. . 2017). The immunosuppression caused by the viral infection allows the bacterial agent to migrate and colonize the lower respiratory tract, leading to pulmonary involvement and a serious infection. Among the most important bacterial agents we can mention Mannheimia haemolytica, Pasteurella multocida, Histophilus somni (Jared et al. 2010).

The development of symptoms can occur in an acute or chronic clinical form. In the chronic form, the animals present the subclinical form. In this, the animals are apparently healthy, and may present mild mucoid or mucopurulent oronasal discharge (Assis-Brasil et al. 2013). The clinical signs of the acute form include dyspnea, apathy, weight loss, tremors, bruxism, noisy breathing, tachypnea, serous or mucopurulent nasal discharge, fever, productive cough, recumbency and death (Assis-Brasil et al., 2013).

Therefore, the objective of the present study was to verify the presence through serological and/or antigenic detection of etiological agents causing BRD, such as Bovine Hespesvirus Type 1 (BoHV-1), Bovine Viral Diarrhea Virus, Parainfluenza Virus Type 3 (BPI-3) in blood and nasal swab samples from 100 unvaccinated bulls from four properties in the municipality of Paranatinga sent for a feedlot located in the municipality of Ipiranga do Norte, MT.

II. MATERIAL AND METHODS

The present study was carried out in a rural property located in the municipality of Ipiranga do Norte MT that receives beef cattle for finishing, with a confined population of approximately five thousand animals per month. Twenty-five bulls were randomly selected from each of the four selected properties in the municipality of Paranatinga, MT. The animals that participated in the study were clinically evaluated upon arrival at the confinement, and then identified with earrings. All animals were intact males with a mean age of 15 months and mean entry weight of 353.61 kg. None of the properties evaluated performed immunization of the animals against the mentioned agents evaluated in this study.

For diagnostic procedures on the day of arrival of the animals, blood samples were collected by coccygeal venipuncture, in addition to a double nasal swab from each of the animals involved in the study. The samples were identified and sent under refrigeration in isothermal boxes to the Laboratory of Infectious Diseases at UFMT Sinop where the samples were processed.

The blood was desorbed under centrifugation and the serum was stored in double aliquots in 1.5 mL microtubes and kept under refrigeration between +2 and +8ºC. The swabs were identified and kept under refrigeration between +2 and +8ºC.

The day after collection, the blood samples and swabs were taken to the Biological Institute of São Paulo, where they were re-identified and sent to the Bovine Viral Diseases Laboratories for analysis. Serum samples were evaluated by the virus neutralization technique for the presence of antibodies against Bovine Herpesvirus Type 1, Viral Diarrhea Virus and Parainfluenza Virus Type 3. From the nasal swab, Viral Isolation techniques were performed to search for BoHV-1 and BPI3 and subsequent confirmation by the Real Time Polymerase Chain Reaction (qPCR) for BoHV-1 as well as the ELISA for antigenic detection of BVDV from serum and swab samples.

For the virus neutralization (VN) test, 96-well, flat-bottomed microtiter plates were used. Column one of the test plate was used to control cells, column two to control the toxicity of each serum and in columns three to 12 the samples were serially diluted, on a logarithmic basis 2 from a 1:2 dilution to 1:1024 for BPI3 and BoHV-1; for BVDV 1:10 to 1:5.120, using MEM medium as diluent. For validation of the test, standard negative, weak positive and positive serum with known antibody titers were included. The plates were incubated for one hour in an oven at 37ºC with 5% CO2, after which they received 50μL of MDBK (Madin-Darby Bovine Kidney) cell suspension, at a concentration of 3 x 105 cells/mL for BPI3 VNs and BVDV In the case of BoHV-1, the incubation took place for 18 to 24 hours, after which 100μL of MDBK cell suspension was added, at a concentration of 3 x 105 cells/mL.

Infectivity was indicated by the visible cytopathic effect on the cell monolayer on plates, under an inverted microscope, after four days of incubation at 37ºC and 5% CO2. The antibody titer was expressed as the highest serum dilution that completely inhibited infectivity in both wells of each dilution, with the lowest dilution detected by the BPI3 and BoHV-1 test being 1:2 and BVDV 1:10, whose titer was was calculated and expressed in log10.

Samples with titers equal to or greater than 0.3 log10 were considered reagents. The test was validated when the cells from the wells destined to control cells remained intact, the cells from the wells to control the toxicity of the sera also remained unaltered, that is, they did not present a toxic effect, and the negative, weak positive and positive control sera showed the expected results. Regarding dose control, the test was validated when the infecting dose was between 300 and 3000/mL, and if in the viral re-titration the titer obtained presented a variation of only ±03log10 when compared to the titer of the stock solution, calculated according to the method by Reed and Muench (1938).

In the viral isolation, the samples were first processed, where each swab sample was subjected to vortex agitation and the swab was pressed against the wall of the tube for total removal of the secretion. Each sample was kept in a -20ºC freezer until laboratory analysis. The previously processed swab was thawed and subjected to viral isolation in 24-well cell culture plates, previously prepared and containing a monolayer of MDBK (Madin-Darby Bovine Kidney) cells at a concentration of 2x105 cells/mL. After 24 hours of cell growth, the MEM medium was removed, the monolayer washed and 200 µL of the sample suspension was inoculated into each well of the plate.

After one hour of incubation in a controlled oven at 37ºC containing 5% CO2, the inoculum was discarded, the monolayer washed with MEM medium and then 1 mL of maintenance medium (MEM medium containing 2% fetal bovine serum and 1% antibiotic solution). The monolayer was observed under an inverted optical microscope and monitored daily for seven days to visualize the cytopathic effect (CPE). This procedure was repeated two more times, with an interval of seven days each. Samples that showed CPE were subjected to quantitative real-time PCR for BoHV-1 (qPCR) to confirm the diagnosis, according to OIE (2019). Samples were considered negative after three passages without the presence of CPE (OIE 2019). To detect BVDV in the swab, the IDEXX® BVDV Ag kit was used, according to the manufacturer's instructions.

This study was approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Mato Grosso under number 28108.713904/2015-91.

III. RESULTS AND DISCUSSION

Immediately after unloading the animals on the property, a clinical evaluation of the animals was carried out, and no animal with clinical symptoms related to the Bovine Respiratory Disease Complex was found. After laboratory analysis of the collected samples, the presence of the three viral agents or antibodies against them was verified in animals from the four properties evaluated.

Regarding the presence of anti-Parainfluenza-3 (PI-3) virus antibodies, only four animals were not reactive by the virus neutralization technique, where all properties showed seroreactive animals. The frequency of 96% of reagent animals corroborates the findings in several studies in Brazil. Candeias and Ribeiro (1970) detected 84.1% of seropositive animals in the state of São Paulo. Wizigmann et al. (1972) found a frequency of approximately 97% in bovine serum samples positive for the presence of anti-PI-3 antibodies. Viral circulation in sheep was also verified, as reported in the studies by Gonçalves et al. (2003) and Gonçalves et al. (2009), respectively in the states of Rio Grande do Sul and São Paulo.

Abroad, several studies also report high rates of occurrence of seroreactive animals. Durham and Hassard (1990) found prevalence rates of 93.9% and 99.7% for focus animals and farms in Canada. In France, Vallacher and Hagglung (2006) detected anti-PI-3 antibodies in 100% of the cows tested. More recently, Murray et al. (2017) reported that PI-3 was the most isolated viral agent from cases of animals with respiratory disease in Ireland. Similarly Callaby et al. (2016) verified the presence of the aforementioned agent as one of the main causative agents of BRC in wild cattle in Kenya.

In general, most studies report a high occurrence of seroreactive animals, however with almost all animals without any clinical symptoms. This fact was also verified in the current study. According to Gonçalves et al. (2003), PI-3 causes a mild disease with mild respiratory conditions with many animals presenting in the subclinical form.

Regarding the serological diagnosis of the causative agent of Infectious Bovine Rhinotracheitis (IBR), of the 100 animals evaluated in the present study, all had anti-BoHV-1 antibodies by the virus neutralization test. When the viral isolation technique was used, the agent was detected in nine animals, which were confirmed by the qPCR technique.

These results confirm what has already been mentioned by other authors, that the best technique for diagnosing the agent is serological as opposed to antigenic, given the possibility of Herpesviruses being in a latency phase, making it difficult to detect the agent outside the viremia phase (Takiuchi et al. 2001; Flores et al. 2012).

In relation to the frequency verified in the animals of Paranatinga (MT), the result obtained presented the highest rate of occurrence among the studies verified in the country. Richtzenhain et al. (1999) carried out the largest study in Brazil on the prevalence of BoHV-1. In this study, 1992 herds were evaluated in all Brazilian states and a total of 21062 animals. According to the authors, 94.7% of the herds and 64.3% of the samples were positive for the

virus neutralization technique, demonstrating a wide viral circulation throughout the country. Similarly, Dias et al. (2013) more recently performed a retrospective study of 14803 samples of unvaccinated females from 2018 herds in the state of Paraná. In this study, a prevalence of 59.0% and 71.3% of positive animals and herds was verified, respectively.

In the Midwest region, in different studies carried out in the state of Goiás, different authors evaluated the occurrence of BoHV-1. According to Barbosa et al. (2005), the observed prevalence was 98.5% of the properties, while 51.9% of the 6932 animals were seroreactive. Corroborating the findings of this study, Vieira et al. (2006) found a prevalence closer to that found in the present study. According to these authors, 83% of the samples were seropositive, while almost all the properties (96.7%) were foci of the agent.

The isolation of the agent, associated with confirmation by qPCR, reports the high occurrence of the agent in unvaccinated herds. However, possibly these are strains of low pathogenicity since no animals with clinical aspects of diseases caused by BoHV-1 were observed, or the subclinical picture was due to the humoral memory response of the animals.

Regarding the last viral agent evaluated, 62% of the animals had anti-BVDV antibodies at the time of arrival at the Feedlot, and it was observed that all properties had reactive animals. Among the non-reactive animals, none of them showed the presence of a persistently infected animal by the ELISA technique.

In population studies carried out in Brazil on Bovine Viral Diarrhea, Chaves et al. (2010) found that 61.5% of animals were seropositive to the virus neutralization technique, while 95% of properties in the Amazon region of Maranhão had seroreactive animals. Brito et al. (2010), in the state of Goiás, found a prevalence of 64% of seropositive animals and 88.3% of focus properties. More recently, Fernandes et al. (2016) carried out a study in herds in the state of Paraíba. In this study, prevalences of 65.5% and 39.1% of seropositive herds and animals were observed, respectively.

The results obtained in the present study are similar to most of the works found in the literature. The circulation of BVDV is common in the national herd, and these agents are possibly of low pathogenicity, thus not causing animals with clinical signs of the disease.

According to the results obtained, it was possible to verify the wide circulation of several agents with great potential for pathogenicity in animals from properties in the municipality of Paranatinga, Mato Grosso. Despite the high occurrence of seropositive animals for BoHV-1, BVDV and PI-3, none of them showed any change in the clinical examination upon arrival at the property.

According to Callaby et al. (2016), there is a high correlation between the joint occurrence of the three pathogens evaluated in the present study in animals, with their co-circulation in cattle herds being common.

Therefore, due to the productive pressure in confinement conditions, sanitary management is of great importance in these animals raised intensively with a focus on nutrition and health. Associated with this, it is recommended to cattle producers with the objective of confinement to prevent with polyvalent vaccines aimed at immunizing animals from an early age to avoid damage in cases of outbreaks of these diseases.

IV. CONCLUSION

The results of this study pointed to the circulation of viruses of relevance to bovine health, especially in intensive production environments such as feedlots. The detection of antibodies against the agents, as well as the specific verification of the pathogens increases the need for epidemiological surveillance and prevention of the agents related to the Complete Respiratory Diseases of Cattle from the properties related to the reproduction and production of calves. In this way, the use of vaccines would be an efficient way to avoid possible contamination and the development of clinical conditions in the adult phase of the animal.

REFERENCES

[1] Brasil, N. D. A., Hinnah, F. L., Fiss, L., Sallis, E. S., Grecco, F. B., Ladeira, S. R., ... & Schild, A. L. (2013). Doenças respiratórias em bezerros na região sul do Rio Grande do Sul: estudo retrospectivo de 33 surtos. *Pesquisa Veterinária Brasileira*, 33, 745-751.

[2] Silva, B. P. e, Soares, L. B. F., Macêdo, A. A. de, Oliveira, J. M. B. de, Aragão, B. B., Nascimento, S. A. do, & Pinheiro Júnior, J. W. (2020). Soroprevalência e fatores de risco associados ao herpesvírus bovino tipo 1 e ocorrência da infecção pelo vírus da diarreia viral bovina em vacas leiteiras no estado de Pernambuco. *Medicina Veterinária (UFRPE)*, 13(3), 399–405. https://doi.org/10.26605/medvet-v13n3-3302

[3] BRASIL. Ministério da Agricultura Pecuária e Abastecimento. 2020. Disponível em <https://agenciadenoticias.ibge.gov.br/agencia-noticias/2012-agencia-de-noticias/noticias/16994-rebanho-de-bovinos-tem-maior-expansao-da-serie-historica.html> Acesso em 05 de dezembro de 2021.

[4] Brito, W. M. E. D. de, Alfaia, B. T., Caixeta, S. P. de M. B., Ribeiro, A. C. C., Miranda, T. de M. T., Barbosa, A. C. V. C., Barthasson, D. L., Linhares, D. C., & Faria, B. O. (2010). Prevalência da infecção pelo Vírus da Diarréia Viral Bovina (BVDV) no Estado de Goiás, Brasil. *Revista De*

Patologia Tropical, 39(1), 07–20. https://doi.org/10.5216/rpt.v39i1.9494

[5] Callaby, R., Toye, P., Jennings, A., Thumbi, S. M., Coetzer, J. A., Conradie Van Wyk, I. C., Hanotte, O., Mbole-Kariuki, M. N., Bronsvoort, B. M., Kruuk, L. E., Woolhouse, M. E., & Kiara, H. (2016). Seroprevalence of respiratory viral pathogens of indigenous calves in Western Kenya. *Research in veterinary science*, 108, 120–124. https://doi.org/10.1016/j.rvsc.2016.08.010

[6] Candeias, J.A.N. & Ribeiro, L.C. (1968). Anticorpos inibidores da hemaglutinação para o vírus parainfluenza 3 (Ha-1), em gado bovino. *Revista de Saúde Pública*. 2(2):180-185. DOI: 10.1590/S0034-89101968000200004

[7] Chaves, N.P., Pereira, H.M., Sousa, V.E., Santos, H.P. & Bezerra, D.C. (2010). Frequência de anticorpos e fatores de risco para a infecção pelo vírus da diarreia viral bovina em fêmeas bovinas leiteiras não vacinadas na região Amazônica Maranhense, Brasil. *Ciência Rural*, 40(6),1448-1451. [fecha de Consulta 19 de Septiembre de 2022]. ISSN: 0103-8478. Disponible en: https://www.redalyc.org/articulo.oa?id=33117724028

[8] Dias, J. A., Alfieri, A. A., Ferreira-Neto, J. S., Gonçalves, V. S., & Muller, E. E. (2013). Seroprevalence and risk factors of bovine herpesvirus 1 infection in cattle herds in the state of Paraná, Brazil. *Transboundary and emerging diseases*, 60(1), 39–47. https://doi.org/10.1111/j.1865-1682.2012.01316.x

[9] Durham, P. J., & Hassard, L. E. (1990). Prevalence of antibodies to infectious bovine rhinotracheitis, parainfluenza 3, bovine respiratory syncytial, and bovine viral diarrhea viruses in cattle in Saskatchewan and Alberta. *The Canadian veterinary journal*, 31(12), 815–820.

[10] Earley, B., Buckham Sporer, K., & Gupta, S. (2017). Invited review: Relationship between cattle transport, immunity and respiratory disease. *Animal*, 11(3), 486–492. https://doi.org/10.1017/S1751731116001622

[11] Edwards T. A. (2010). Control methods for bovine respiratory disease for feedlot cattle. *The Veterinary clinics of North America. Food animal practice*, 26(2), 273–284. https://doi.org/10.1016/j.cvfa.2010.03.005

[12] Fernandes, L. G., Nogueira, A. H., De Stefano, E., Pituco, E. M., Ribeiro, C. P., Alves, C. J., Oliveira, T. S., Clementino, I. J., & de Azevedo, S. S. (2016). Herd-level prevalence and risk factors for bovine viral diarrhea virus infection in cattle in the State of Paraíba, Northeastern Brazil. *Tropical animal health and production*, 48(1), 157–165. https://doi.org/10.1007/s11250-015-0937-x

[13] FLORES E. (2012) *Virologia veterinária: virologia geral e doenças víricas*. 2nd ed. Editora da UFSM, Santa Maria.

[14] Franco, A.C., Oliveira, M., Spilki, F., Roehe, P.M., Chiminazzo, C., Gonçalves, D. (2003). Isolamento do vírus Parainfluenza bovino tipo 3 no Rio Grande do Sul, Brasil. *Ciência Rural*, 33(5),953-956. https://www.redalyc.org/articulo.oa?id=33133525

[15] Gonçalves, R. C., Silva, A. A. da, Ferreira, D. O. L., Marcondes, J., Pituco, E. M., & Dias, A. (2009). Ocorrência do vírus parainfluenza-3, vírus respiratório sincicial, vírus da diarréia viral bovina e herpevírus tipo 1 em rebanhos ovinos da região de Botucatu-SP. *Ciência Animal Brasileira*, 1, 563–568. https://revistas.ufg.br/vet/article/view/7859

[16] Griffin D. (2014). The monster we don't see: subclinical BRD in beef cattle. *Animal health research reviews*, 15(2), 138–141. https://doi.org/10.1017/S1466252314000255

[17] Guzman, E., & Taylor, G. (2015). Immunology of bovine respiratory syncytial virus in calves. *Molecular immunology*, 66(1), 48–56. https://doi.org/10.1016/j.molimm.2014.12.004

[18] Taylor, J. D., Fulton, R. W., Lehenbauer, T. W., Step, D. L., & Confer, A. W. (2010). The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors?. *The Canadian veterinary journal*, 51(10), 1095–1102.

[19] MATO GROSSO, Instituto Matogrossense de Economia Agropecuária. 2022. Relatório Técnico 05 de Maio de 2022. Disponível em < http://www.imea.com.br/upload/publicacoes/arquivos/08052017201905.pdf>. Acesso em 12 de maio de 2022.

[20] Murray, G. M., More, S. J., Sammin, D., Casey, M. J., McElroy, M. C., O'Neill, R. G., Byrne, W. J., Earley, B., Clegg, T. A., Ball, H., Bell, C. J., & Cassidy, J. P. (2017). Pathogens, patterns of pneumonia, and epidemiologic risk factors associated with respiratory disease in recently weaned cattle in Ireland. *Journal of veterinary diagnostic investigation*, 29(1), 20–34. https://doi.org/10.1177/1040638716674757

[21] OIE Terrestrial manual 2019. International Animal Health Code. Manual of standards. Disponível em: <http://www.oie.int/fileadmin/Home/esp/Health_standards/tahm/2.04.13.%20Rinotraqueitis%20infecciosa%20bovina.pdf> Acesso em: 15 mar. 2019.

[22] L.J. REED, H. MUENCH (1938) A simple method of estimating fifty per cent endpoints. *American Journal of Epidemiology*, 27(3), 493–497, https://doi.org/10.1093/oxfordjournals.aje.a118408

[23] Richtzenhain, L. J., Barbarini, O., Umehara, O., De Gracia, A. S., Cortez, A., Heinemann, M. B., & Ferreira, F. (1999). Rinotraqueíte infecciosa 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*, 66, 83-88.

[24] Takiuchi E., Médici K.C., Alfieri A.F. & Alfieri A. A. (2003). Otimização da reação em cadeia pela polimerase (Semi Nested-PCR) para a detecção do herpesvírus bovino tipo 1 em fragmentos de órgãos fetais e em semen de bovinos naturalmente infectados. *Semina, Ciências Agrárias* 24(1):43-56.

[25] Valarcher J.F. & Hägglund S. (2006). Viral respiratory infections in cattle. In: Proceedings of XXIV World Buiatrics Congress. Nice, France. https://pdfs.semanticscholar.org/ab14/cd3e578fdd97c7e314a6470ad67ed60c892f.pdf

[26] Vieira S., Brito W.M.E.D., Souza W.J., Alfaia B.T. & Linhares D.C.L. (2003). Anticorpos para o herpesvírus bovino 1 (BHV-1) em bovinos do estado de Goiás. *Ciência Animal Brasileira*. 4(2):131-137.

[27] Wizigmann G., Vidor T. & Ricci Z.M.T. (1972). Investigações sorológicas sobre a ocorrência e incidência dos vírus da diarréia a vírus-enfermidade das mucosas dos bovinos, no estado do Rio Grande do Sul. *Boletim do Instituto de Pesquisas Veterinárias Desidério Finamor* 1:2-58.