ijvm.2019.283696.1004998

Iranian Journal of Veterinary Medicine
Volume 14- Issue 01

Original Article
Online ISSN : 2252-0554

Molecular and Serological Evaluation of Bovine Leukemia Virus in Water Buffaloes of Southern Iran

Atefeh Esmailnejad, Hamideh Najafi*, Yousuf Torfi

[1] *Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran*

Abstract

BACKGROUND: Bovine leukemia virus (BLV) is an oncogenic *deltaretrovirus* causing a persistent lifelong infection of B lymphocytes. In addition to the domestic cattle, the virus can also infect water buffaloes.

OBJECTIVES: Recent investigations have demonstrated the increasing prevalence of BLV infection among cattle population in Iran. Large populations of water buffaloes are also kept in different parts of Iran for milk and meat purposes. Considering economic losses induced by BLV infection in buffalo and more importantly the role of this species in virus epidemiology, the present study has investigated the BLV infection in Iranian water buffalo population.

METHODS: Seroprevalence and occurrence of BLV was investigated in water buffalo population (n=100) in Khuzestan province, Southwest Iran by ELISA and nested PCR, targeting gp51 region in the *env* gene.

RESULTS: In total, 52 samples were seropositive and represented the antibodies against BLV gp51 protein in ELISA test. Forty-seven out of 52 seropositive samples were confirmed by nested PCR.

CONCLUSIONS: Such a high rate of BLV infection in water buffaloes is an alarming issue for both its economic impact due to the production losses and more importantly the epidemiological aspects in which the virus circulation among different host species will complicate the control and prevention strategies.

KEYWORDS: BLV; ELISA; epidemiology; Nested PCR; water buffalo

Correspondence
Hamideh Najafi, Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
Tel: +98(71) 36138673, Fax: +98(71)32286940, Email: h.najafi@shirazu.ac.ir
Received: 2019-06-17
Accepted: 2019-07-28

Copyright © 2020. This is an open-access article distributed under the terms of the Creative Commons Attribution- 4.0 International License which permits Share, copy and redistribution of the material in any medium or format or adapt, remix, transform, and build upon the material for any purpose, even commercially.

How to Cite This Article

Esmailnejad, A., Najafi, H., & Torfi, Y. (2020). Molecular and Serological evaluation of Bovine Leukemia Virus in Water Buffaloes of southern Iran. Iranian Journal of Veterinary Medicine, 14(1),

Introduction

Enzootic bovine leukosis is an economically important disease of cattle which usually causes clinically silent infections. The disease occurs worldwide, infecting cattle and, regionally, water buffalo. Bovine leukemia virus (BLV) as the etiologic agent of the disease is a single-stranded positive sense RNA delta-retrovirus, from the Retroviridae family. While about 70% of BLV infected cattle remain asymptomatic (Aleukemic form), approximately 30% develop persistent lymphocytosis (PL) with polyclonal expansion of B lymphocytes. Only 1-3% of infections progress to the form of multicentric lymphosarcoma (LS) (Maclachlan and Dubovi, 2010; Murakami et al., 2018; Okagawa et al., 2018; Ruggiero et al., 2018). Although few of the BLV infected cattle actually develop clinical signs of the disease, the infection poses economic losses relating to the decreased longevity, reduced milk production and compromised immune system. Rat, rabbit, pig, chicken, goat and sheep can be experimentally infected by the virus, but cattle and water buffalo as the natural hosts of BLV are particularly important to epidemiological investigations (Maclachlan and Dubovi, 2010; Kuczewski et al., 2018).

Serological assays including enzyme-linked immunosorbent assay (ELISA) as the most common detection methods for identifying BLV infected animals are rapid and inexpensive. However, as antibody response may not be induced until 14 weeks after infection, they are not always efficient. It has been demonstrated that BLV infected cattle could be transiently seronegative in some conditions such as early infections, per parturient state and co-infection with other viruses (Roberts et al., 1988). In such situations while the animal is viremic and able to transmit the virus, the serology result is negative (Kuckleburg et al., 2003). On the other hand, cross reactivity with other members of Retroviridae family could cause false positive results. Molecular methods such as PCR and more sensitive; nested-PCR have provided reliable diagnosis in early stages even in young animals and attracted much attention in recent years (Gonzalez et al., 2001; Benavides et al., 2017).

The world population of water buffalo (*Bubalus bubalis*) is approximately 168 million head; more than 95 percent are located in Asia (FAO, 2017). Buffalo is among the most productive domestic animals and has been recognized for thousands of years as an efficient draught species. As a result of unique conversion capacity, buffalo is considered as a source of high quality meat which can be produced more cheaply than cattle. Buffalo can thrive under different climatic conditions and adapt to all save arid or arctic environments. Under proper management, the animal adjusts readily to intensive agricultural systems in high temperate climates (Cockrill, 1981).

The population of water buffalo in Iran was recorded approximately 127000 head in 2017 (FAO, 2017). Iranian buffaloes are commonly maintained in the south, north, northwest and southwest areas mainly for meat and milk purposes, playing a key role in the economy of rural families (Naserian and Saremi, 2007; Oryan et al., 2010). Although BLV is relatively prevalent in Iranian cattle, there is no information on its prevalence in water buffalo populations as another natural host of the virus. The aim of the study described here was to evaluate the occurrence and seroprevalence of BLV infection among water buffalo population in Khuzestan prov-

ince, Southwest Iran.

Materials and methods

Sampling and DNA extraction

The study was performed on a total of 100 Iranian water buffalo (≤ 3 years old) referred to the slaughterhouse in Khuzestan province, southwest Iran from June 2016 to December 2017. Blood samples were collected from the cephalic veins and sera were separated and stored at –20 °C until serological assay. Blood samples with ethylene diamine tetra acetic acid (EDTA) were also collected for molecular analysis. DNA extraction from peripheral blood leukocytes was performed using a Genomic DNA Extraction Kit (Genet Bio, Seoul, Korea). The study was approved by Shiraz University, Policy on Animal Care and Use.

Enzyme-linked immunosorbent assay (ELISA)

For detection of antibodies against Bovine leukemia Virus in buffaloes' sera samples, the Enzyme-linked immunosorbent assay (ELISA) test was performed. Anti-gp51 BLV antibodies were detected using a commercial competitive ELISA kit (IDVet, France) according to the manufacturer's instructions. The sample/negative control (S/N) percentage was calculated by the formula:

S/N% = [ODsample] / [ODNC] × 100.

Sera with an S/N% ≤ 50 were considered negative; samples with S/N% between 50 and 60 were considered doubtful; and samples with S/N% ≥ 60% were considered positive.

Detection of BLV provirus

Proviral BLV DNA was detected by nested polymerase chain reaction of the BLV env gene. Two pairs of primers were used for PCR amplification as follows: External primers 5'- TCTGTGCCAAGTCTCCCAGATA-3' and 5'- AACAACAACCTCTGGGAAGGGT-3' as well as internal primers 5'- CCCACAAGGGCGGCGCCGGTTT-3', and 5'-GCGAGGCCGGGTCCAGAGCTGG-3' which resulted in the amplification of 598 and 444 bp DNA fragments in the gp 51 region of the env gene, respectively.

Amplification was performed in a final volume of 25 µL containing 20 ng genomic DNA, 100 ng of each primer, 1.5 mM MgCl2, 200 µM of each dNTP, 50 mM KCl and 20 mM Tris–HCl, and 1 U/µL Taq DNA polymerase (CinaClon, Iran). The thermal cycling profile was 1 cycle of 94 °C for 9 min, 40 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C (for external primers) or 70 °C (for internal primers) for 30 s and extension at 72 °C for 60 s, with a terminal extension of 4 min at 72 °C. The second round of PCR was carried out using 1 µl of the first-round product and the same concentration of reagents and corresponding primers. The amplified PCR products were detected using electrophoresis on 1% agarose gel and visualized by ultraviolet (UV) light. BLV positive control was kindly provided by Tehran University, Iran. DNA sample prepared from confirmed BLV-seronegative cattle was also used as negative control.

Statistical analysis

The data was presented in a 2×2 table and analyzed for sensitivity, specificity, and predictive values using the following formulas:

Sensitivity= number of true positives/ (number of true positives+ number of false negatives)

Specificity= number of true negatives/ (number of true negatives+ number of false positives)

Positive predictive value= number of true positives/ (number of true positives+ number of false positives)

Table 1. Sensitivity and specificity, positive and negative predictive values the ELISA against PCR

ELISA \ PCR	+	_	Total
+	47	5	52
_	0	48	48
Total	47	53	100

Negative predictive value= number of true negatives/ (number of true negatives+ number of false negatives).

Results

Out of 100 samples collected from buffaloes, 52 samples (52%) had antibodies against BLV gp51 protein, measured by ELISA technique. Nested PCR of gp 51 region in the env gene also revealed 47 positive specimens (47%), giving 598 and 444 bp DNA fragments.

ELISA yielded 52 positive suspects, 5 of them were PCR negative. According to the Table 1, the sensitivity, specificity, positive and negative predictive values of the ELISA were 100%, 90.56%, 90.38% and 100%, respectively.

Discussion

Of the 194.29 million buffaloes in the world, 179.75 million (92.52%) are water buffaloes, primarily located in Asia (Hamid et al., 2016). Water buffaloes are distributed in different parts of Iran, posing an indispensable source of human needs including meat and milk. Unfortunately, few studies have been conducted to investigate the viral pathogens infecting water buffaloes, especially viruses that cause clinically silent infections while acting as a source of virus transmission. Results of the present study showed the BLV seroprevalence of 52% by ELISA and 47% by nested PCR in the water buffalo population in the southwest of Iran. To the best of our knowledge, this is the first research that attempted to study the BLV infection in buffalo population in Iran.

Limited studies have investigated the seroprevalence of BLV in buffalo population all around the world. Moreover, different serological and molecular methods with different sensitivity and specificity levels have been applied to determine the disease status in buffalo populations, including ELISA, agar gel immunodiffusion (AGID), immunofluorescence (IF), viral neutralization test and PCR. A study conducted on 370 water buffaloes in Pakistan reported 0.8 % prevalence of anti-BLV antibodies by immunodiffusion test (Meas et al., 2000b). Mingala et al. also reported 27.6% prevalence in Philippines water buffaloes obtained by nested PCR (Mingala et al., 2009). Oliveira et al. investigated the seroprevalence of BLV in 315 buffaloes from different regions in Brazil by commercial ELISA, AGID and PCR methods. In spite of detection of antibodies against the whole virus in 24.44% of serum samples by ELISA, the immunodiffusion test and PCR technique targeting the env and tax genes revealed negative results (De Oliveira et al., 2016). Investigation of BLV in cattle has also demonstrated that ELISA can give false positive results, when compared with direct detection test such as PCR. The prev-

alence of BLV in cattle in Thailand ranged from 5.3% to 87.8% as determined by nested PCR, and 11.0% to 100% by ELISA (Lee et al., 2016). Benavides et al also detected 31 positive cows by Indirect ELISA, while 27 sera were confirmed by nested PCR (Benavides, 2017). It can be concluded that serological tests like ELISA that are usually used for detection of BLV in cattle can produce false-positive results in buffalo. Therefore, a combination of serology with molecular techniques may represent more accurate data (De Oliveira et al., 2016). An immunodiffusion test had also failed to demonstrate infection among 42 muscle water buffaloes in Cambodia (Meas et al., 2000a).

While there is scarce information regarding BLV infection in Iranian water buffaloes, several studies have investigated the BLV status in cattle and different prevalence rates have been reported. Initial inspection in Iran revealed low seroprevalence of BLV ranging between 0.5 and 5.7% in cattle (Mohammadi et al., 2011). Subsequent studies which used more sensitive serological assays including ELISA disclosed higher rates of infection. Between July 2006 and April 2007, a total of 882 female dairy cattle were tested for some viral diseases and BLV seroprevalence was reported to be 16.2% (Nikbakht et al., 2014). Among 429 blood samples collected during 2009 from industrial dairy cattle herds of northeastern provinces of Iran, BLV antibodies were detected in 25.4% of samples (Mousavi et al., 2014). From 137 samples collected from cattle between July 2010 and January 2011, 29.9% had antibodies against BLV (Mohammadi et al., 2011). BLV infection was also highly prevalent (81.9%) among cattle population in central region of Iran (Isfahan province) (Morovati et al., 2012). Brujeni et al. detected BLV in 17% of 143 tested Holstein cattle using nested PCR (Brujeni et al., 2010). In another study conducted on cattle, sheep and camel populations, the prevalence rates of 22.1%, 5.3 % and 0% have been reported respectively, using nested PCR technique (Nekoei et al., 2015). The only study that assessed BLV infection in buffaloes was conducted in Ahvaz which detected one positive sample within the 529 slaughtered buffaloes (Hajikolaei et al., 2015). In conclusion, considering the high prevalence of BLV infection in cattle population, the role of buffalo as another natural host of BLV in transmitting the disease is quite prominent. High prevalence of BLV observed in Iranian water buffaloes is an alarm that these animals are probably an important source of infection, leading to the virus circulation among different hosts.

Acknowledgments

This work was approved and funded by the Research Committee at Shiraz University, School of Veterinary Medicine, Shiraz, Iran [grant number 96GRD1M340870].

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

Benavides, B., Muñoz, S., Ceriani, C. (2017). Molecular analysis of a fragment of bovine leukemia virus env gene by Nested-PCR in dairy cows from Pasto, Nariño. Rev Med Vet, 33, 67-75. http://dx. doi. org/10.19052/mv. 4054.

Brujeni, G. N., Poorbazargani, T. T., Nadin-Davis, S., Tolooie, M., Barjesteh, N. (2010). Bovine immunodeficiency virus and bovine leukemia virus and their mixed infection in Iranian Holstein cattle. J Infect Dev Ctries, 4, 576-579.https://doi.org/10.3855/jidc.711. PMID: 21045371.

Cockrill, W. R. (1981). The water buffalo: a review. Br Vet J, 137, 8-16. https://doi.org/10.1016/S0007-1935(17)31782-7. PMID: 7013899.

De Oliveira, C. H., Resende, C. F., Oliveira, C. M., Barbosa, J. D., Junior, A. A. F., Leite, R. C. et al. (2016). Absence of Bovine leukemia virus (BLV) infection in buffaloes from Amazon and southeast region in Brazil. Prev Vet Med, 129, 9-12. https://doi.org/10.1016/j.prevetmed.2016.05.002. PMID: 27317318.

FAO (2017). Food and Agriculture Organization of the United Nations. FAOSTAT database.

Gonzalez, G. C., Johnston, J. B., Nickel, D. D., Jacobs, R. M., Olson, M., Power, C. (2001). Very low prevalence of bovine immunodeficiency virus infection in western Canadian cattle. Can J Vet Res, 65, 73-76. PMID: 11227201.

Hajikolaei, M. R. H., Seifi-Abadshapouri, M. R., Changizi, F. (2015). Serological study of Bovine leukemia virus (BLV) infection in slaughtered buffalo in Ahvaz. Iranian Journal of Veterinary Clinical Sciences, 9.

Hamid, M., Ahmed, S., Rahman, M., and Hossain, K. (2016). Status of buffalo production in Bangladesh compared to SAARC countries. Asian J Anim Sci, 10, 313-329. https://doi.org/10.3923/ajas.2016.313.329.

Kuckleburg, C. J., Chase, C. C., Nelson, E. A., Marras, S. A., Dammen, M. A., Christopher-Hennings, J. (2003). Detection of bovine leukemia virus in blood and milk by nested and real-time polymerase chain reactions. J Vet Diagn Investig, 15, 72-76. https://doi.org/10.1177/104063870301500117. PMID: 12580302.

Kuczewski, A., Orsel, K., Barkema, H. W., Kelton, D. F., Hutchins, W. A., van der Meer, F. J. (2018). Evaluation of 5 different ELISA for the detection of bovine leukemia virus antibodies. J Dairy Sci, 101, 2433-2437. https://doi.org/10.3168/jds.2017-13626. PMID: 29274963.

Lee, E., Kim, E. J., Ratthanophart, J., Vitoonpong, R., Kim, B. H., Cho, I. S., et al. (2016). Molecular epidemiological and serological studies of bovine leukemia virus (BLV) infection in Thailand cattle. Infect Genet Evol, 41, 245-254. https://doi.org/10.1016/j.meegid.2016.04.010. PMID: 27090024.

Maclachlan, N.J., Dubovi, E.J (2010). Fenner's veterinary virology, Academic press.

Meas, S., Ohashi, K., Tum, S., Chhin, M., Te, K., Miura, K. (2000). Seroprevalence of bovine immunodeficiency virus and bovine leukemia virus in draught animals in Cambodia. J Vet Med Sci, 62, 779-781. https://doi.org/10.1292/jvms.62.779. PMID: 10945301

Meas, S., Seto, J., Sugimoto, C., Bakhsh, M., Riaz, M., Sato, T., et al. (2000). Infection of bovine immunodeficiency virus and bovine leukemia virus in water buffalo and cattle populations in Pakistan. J Vet Med Sci, 62, 329-331. https://doi.org/10.1292/jvms.62.329. PMID: 10770609.

Mingala, C. N., Konnai, S., Cruz, L. C., Onuma, M., Ohashi, K. (2009). Comparative moleculo-immunological analysis of swamp-and riverine-type water buffaloes responses. Cytokine, 46, 273-282. https://doi.org/10.1016/j.cyto.2009.02.006. PMID: 19285880.

Mohammadi, V., Atyabi, N., Nikbakht, B. G. (2011). Seroprevalence of bovine leukemia virus in some dairy farms in Iran. Glob Vet, 7, 305-309.

Morovati, H., Shirvani, E., Noaman, V., Lotfi, M., Kamalzadeh, M., Hatami, A., et al. (2012). Seroprevalence of bovine leukemia virus (BLV) infection in dairy cattle in Isfahan Province, Iran. Trop Anim Health Prod, 44, 1127-1129. https://doi.org/10.1007/s11250-011-0062-4. PMID: 22210288.

Mousavi, S., Haghparast, A., Mohammadi, G., Tabatabaeizadeh, S. E. (2014). Prevalence of bovine leukemia virus (BLV) infection in the northeast of Iran. Vet Res Forum, 5, 135-139. PMID: 25568707.

Murakami, H., Uchiyama, J., Suzuki, C., Nikaido, S., Shibuya, K., Sato, R., et al. (2018). Variations in the viral genome and biological properties of bovine leukemia virus wild-type strains. Virus Res, 253, 103-111. https://doi.org/10.1016/j.virusres.2018.06.005. PMID: 29913249.

Nanda, A. S., Nakao, T. (2003). Role of buffalo in the socioeconomic development of rural Asia: Current status and future prospectus. Anim Sci J, 74, 443-455. https://doi.org/10.1046/j.1344-3941.2003.00138.x.

Naserian, A. A., Saremi, B. (2007). Water buffalo industry in Iran. Ital J Anim Sci, 6, 1404-1405. https://doi.org/10.4081/ijas.2007.s2.1404.

Nekoei, S., Hafshejani, T. T., Doosti, A., Khamesipour, F. (2015). Molecular detection of bovine leukemia virus in peripheral blood of Iranian cattle, camel and sheep. Pol J Vet Sci, 18, 703-707. https://doi.org/10.1515/pjvs-2015-0091. PMID: 26812810.

Nikbakht, G., Tabatabaei, S., Lotfollahzadeh, S., Nayeri Fasaei, B., Bahonar, A., Khormali, M. (2015). Seroprevalence of bovine viral diarrhoea virus, bovine herpesvirus 1 and bovine leukaemia virus in Iranian cattle and associations among studied agents. J Appl Anim Res, 43, 22-25. https://doi.org/10.1080/09712119.2014.883995.

Okagawa, T., Konnai, S., Nishimori, A., Maekawa, N., Goto, S., Ikebuchi, R. (2018). Cooperation of PD-1 and LAG-3 in the exhaustion of CD4+ and CD8+ T cells during bovine leukemia virus infection. Vet Res, 49, 50-62. https://doi.org/10.1186/s13567-018-0543-9. PMID: 29914540.

Oryan, A., Ahmadi, N., Mousavi, S. M. M. (2010). Prevalence, biology, and distribution pattern of Sarcocystis infection in water buffalo (*Bubalus bubalis*) in Iran. Tropl Anim Health Prod, 42, 1513-1518. https://doi.org/10.1007/s11250-010-9601-7. PMID: 20524065.

Roberts, D. H., Lucas, M. H., Wibberley, G., Westcott, D. (1988). Response of cattle persistently infected with bovine virus diarrhoea virus to bovine leukosis virus. Vet Rec, 122, 293-296. PMID: 2837860.

Ruggiero, V. J., Benitez, O. J., Tsai, Y. L., Lee, P. Y. A., Tsai, C. F., Lin, Y. C., et al. (2018). On-site detection of bovine leukemia virus by a field-deployable automatic nucleic extraction plus insulated isothermal polymerase chain reaction system. J Virol Methods, 259, 116-121. https://doi.org/10.1016/j.jviromet.2018.06.008. PMID: 29902491.

ijvm.2019.283696.1004998

Iranian Journal of Veterinary Medicine
Abstracts in Persian Language

Online ISSN 2252-0554

مجله طب دامی ایران، ۱۳۹۸، دوره ۱۴، شماره ۱، ۷۷-۷۰

ارزیابی مولکولی و سرمی ویروس لوسمی گاو در گاومیش‌های جنوب ایران

عاطفه اسماعیل نژاد ، حمیده نجفی*، یوسف طرفی

گروه پاتوبیولوژی، دانشکده دامپزشکی دانشگاه شیراز، شیراز، ایران

(دریافت مقاله: ۲۷ خرداد ماه ۱۳۹۸، پذیرش نهایی: ۰۶ مرداد ماه ۱۳۹۸)

چکیده

زمینه مطالعه: ویروس لوسمی گاو یک دلتاررتروویروس سرطانزا بوده و باعث عفونت مادام‌العمر لنفوسیتهای B می‌گردد. این ویروس نه تنها گاو، بلکه گاومیش را نیز آلوده میسازد.

هدف: مطالعات اخیر حاکی از شیوع فزاینده ویروس لوسمی گاوی در جمعیت گاوهای ایران بوده است. شایان ذکر است جمعیت کثیری از گاومیش‌ها در مناطق مختلف کشور به منظور تامین گوشت و شیر پرورش داده میشوند. با توجه به مضرات اقتصادی ناشی از عفونت ویروس لوسمی گاوی در گاومیش‌ها و مهمتر از آن، نقش این گونه ی دامی در همه گیرشناسی ویروس، مطالعه حاضر به منظور بررسی عفونت ویروس لوسمی گاو در جمعیت گاومیش‌های ایران طراحی شد.

روش کار: شیوع سرمی و میزان وقوع این عفونت ویروسی در ۱۰۰ رأس از جمعیت گاومیش‌های استان خوزستان (واقع در جنوب غربی ایران)، با استفاده از روش‌های الایزا و واکنش زنجیرهای پلیمراز آشیانه‌ای با هدف تکثیر قطعه کد کننده پروتئین gp۵۱ واقع در ژن env تعیین شد.

نتایج: بر اساس نتایج به دست آمده از آزمون الایزا، ۵۲ عدد از نمونه‌ها از نظر سرم شناسی مثبت و حاوی آنتی بادی علیه پروتئین gp۵۱ بودند. ۴۷ عدد از این ۵۲ نمونه دارای نتیجه مثبت در آزمون واکنش زنجیرهای پلیمراز آشیانه‌ای بودند.

نتیجه گیری نهایی: این میزان بالای عفونت ویروس لوسمی گاوی در جمعیت گاومیش‌ها یک مسئله هشداردهنده نه تنها از نظر پیامدهای اقتصادی ناشی از کاهش تولیدات دامی، بلکه از نظر جنبه‌های همه گیر شناسی ویروس بوده که در مورد دوم گردش ویروس بین گونه‌های مختلف میزبان باعث پیچیدگی رهیافت های کنترل و پیشگیری می گردد.

واژه‌های کلیدی:

ویروس لوسمی گاوی، الایزا، همه گیرشناسی، واکنش زنجیرهای پلیمراز آشیانه‌ای، گاومیش

نویسندهٔ مسئول: حمیده نجفی، گروه پاتوبیولوژی، دانشکده دامپزشکی دانشگاه شیراز، شیراز، ایران. ایمیل: *h.najafi@shirazu.ac.ir*