http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (10): 1754-1757, 2004
ISSN 1028-8880

Comparison of dsRNA Profiles of Sudanese Isolates of Epizootic Hemorrhagic Disease of Deer Virus

Salah M.M. Elamin, Tawfig E. Mohammed, Mohammed M. Salih, Mohammed A. Abdalla, Mohamed E.H. Mohamed, Rihab A. Omer, Abdelrahim Karrar and Imadeldin E. Aradaib
Molecular Biology Laboratory, Department of Medicine, Pharmacology and Toxicology, Faculty of Veterinary Medicine, P.O. Box 32, Khartoum North, Sudan

Abstract: The double stranded RNA (dsRNA) genome segments of the Sudanese isolates of epizootic hemorrhagic disease of deer virus (EHDV) were analysed by agarose gel and sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (SDS-PAGE). EHDV serotype 1 and 2 are enzootic in the United States whereas EHDV-4 and an untyped isolate designated EHDV-318 are enzootic in the Sudan. The dsRNA genome segments profiles of the Sudanese EHDV serotypes 4 and EHDV-318 were compared with those of North American EHDV serotypes 1 and 2. Both systems (agarose gel and SDS-PAGE) showed 10 segments for each virus isolate, which is characteristic pattern of EHDV serogroup. The agarose gel showed identical genome profiles for all Sudanese and North American serotypes of EHDV serogroup. However, SDS-PAGE system was able to detect genetic variation between Sudanese and North American EHDV serogroups and among the Sudanese serotypes of EHDV. The results of this study suggested that, the agarose gel electrophoresis could be used as a supportive or complementary method to facilitate tentative diagnosis of EHDV infection in susceptible animal populations. In addition, the SDS-PAGE could also be used to detect genetic diversity among topotypes of EHDV serogroup from the African or North American continents.

Key words: RNA, EHDV, PAGE , Sudan

INTRODUCTION

Epizootic hemorrhagic disease of deer virus (EHDV) is a double stranded RNA virus of the genus Orbivirus in the family Reoviridae[1,2]. EHDV is a serious veterinary problem in North American white-tailed deer, where the virus produces clinical signs and pathological lesions of fatal hemorrhagic infection, resembling those of blue tongue virus (BTV)[3-5]. EHDV may cause an often-subclinical infection in goats and cattle[6-8]. There are at least ten serotypes of EHDV serogroup, distributed world wide, identified by serum neutralization and plaque inhibition test[9]. Of the 10 serotypes of EHDV serogroup, at least EHDV serotypes 4 and EHDV-318 are enzootic in the Sudan[10]. Epizootics of clinical hemorrhagic disease in deer were reported in the Sudan and were thought to be caused by EHDV[11]. Very little information is available about the genetic diversity among the Sudanese serotypes of EHDV serogroup. Like other orbiviruses, EHDV infection in Sudanese breed of goats and cattle is typically asymptomatic and the disease potential of EHDV serotypes remains unknown[4,12]. EHDV has a genome composed of 10 double-stranded RNA (dsRNA) segments. The genome segments code for the viral proteins[1]. There are 3 nonstructural proteins (NS_1, NS_2 and NS_3) and seven structural proteins. The NS proteins are incorporated into the double layer protein coat[13]. The nonstructural proteins are coded for by genome segments 6, 8 and 10, respectively. The major protein of the outer coat, VP_2, coded for by genome segment 2, is associated with serotype specificity and induction of neutralizing antibody. Group specific antigens and genome segments coding for these antigens are being defined. Genome segment 7 codes for VP_7 and is located at the inner protein coat. VP_7 has been acknowledging as serogroup specific protein. Genome segments 1, 3, 4, 6 and 8 of EHDV were found to be highly conserved with more than 90% homology amongst cognate genes of EHDV serogroup[14].

In the present investigation the RNA genome profiles of the Sudanese and North American serotypes of EHDV1 and 2 were analyzed using agarose gel and sodium dodecyl sulphate(SDS) polyacrylamide gel electrophoresis (SDS-PAGE).

Corresponding Author: Imadeldin E. Aradaib, Professor, Molecular Biology Laboratory, Department of Medicine, Pharmacology and Toxicology, Faculty of Veterinary Medicine, P.O. Box 32, Khartoum North, Sudan
Tel: (249).85.316283 Fax: (249)86.312632 E-mail: aradaib@yahoo.com

MATERIALS AND METHODS

Cell culture and virus propagation: The Sudanese EHDV serotypes 4 and 318 were used. The viruses were recovered from sentinel calves at the University of Khartoum Farm. The North American EHDV serotypes 1 and 2 were obtained from Institute of Animal Disease Research Pirbright Laboratory, United Kingdom. Vero cells were prepared in Minimal Essential Medium (MEM) containing 100 units penicillin/ml and 100 mcg streptomycin/mL, 10% tryptose phosphate broth and 10% Fetal Bovine Serum (FBS) that was heat inactivated at 56°C for 30 min. Cell cultures were incubated at 37°C in a humidified incubator until confluent monolayer was obtained (usually 2-3 days).

Virus isolation and identification: Virus isolation procedure was described previously[5]. Briefly, Vero cell monolayers were inoculated with EHDV isolates. The inoculated cell cultures were incubation at 37°C for 1 h, the inoculated cell cultures were supplemented with MEM containing 2% fetal bovine serum (FBS). The cell cultures were again incubated at 37°C and observed daily until cytopathic effect was 80% complete. All cytopathic agents were identified by serum neutralization test[7]. The infectious material was harvested and centrifuged at 3,000 RPM for 30 min and the cell pellet was used for dsRNA extraction.

Nucleic acid extraction: dsRNA was extracted from infected cells. Total nucleic acid was extracted as described previously[4]. Briefly, the cell pellet was lysed in a lysing buffer containing (proteinase K and 10% SDS). The dsRNA was extracted with phenol, ethanol precipitated and partially purified with 10% lithium chloride. The dsRNA is then vaccum dried and resuspended in 100 microliter distilled water and quantified using spectrophotometer at 260 nm wave length.

Agarose gel electrophoresis: The agarose gel electrophoresis and the staining procedure were described in details in the previous report[10]. The dsRNA was used at a concentration of 100 ng and the agarose gel was used at a concentration of 1%.

Polyacrylamide gel electrophoresis (SDS-PAGE): The SDS-PAGE technique and the staining of the gels with silver nitrate were performed as described previously[15]. The dsRNA was used at a concentration of 100 ng and the PAGE was used at a concentration of 10%.

RESULTS

The agarose gel showed identical genome profiles for all genome segments of the Sudanese and the North American serotypes of EHDV serogroup used in this study (Fig. 1). Each EHDV serotype showed 10 bands, which correspond to viral dsRNA genome segments, typical of EHDV serogroup.

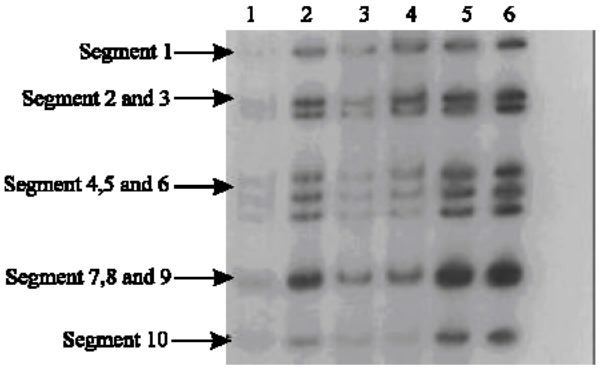

Fig. 1: RNA genome profiles of Sudanese and North American serotypes of EHDV serogroup using 1% agarose gel. Lane 1: EHDV-1; Lane 2: EHDV-2; Lane 3: EHDV-318; Lane 4: EHDV-318; Lane 5: EHDV318; Lane 6: EHDV-4

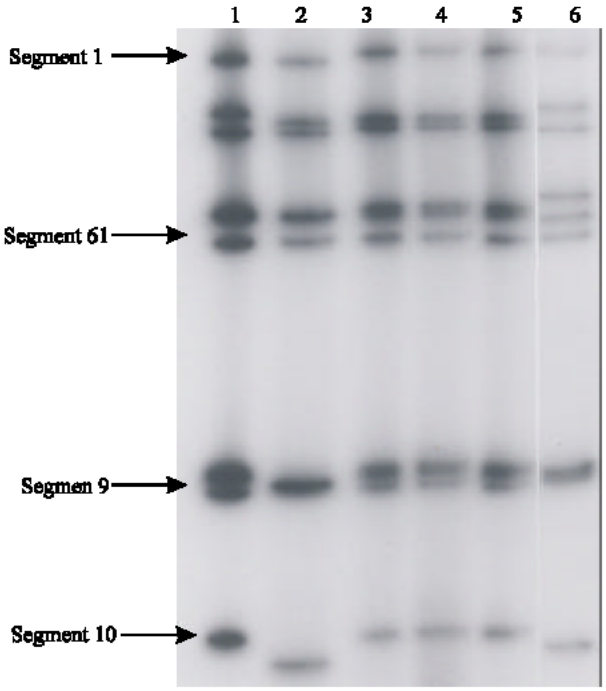

Fig. 2: RNA genome profiles of Sudanese and North American serotypes of EHDV serogroup using 10% SDS-PAGE. Lane 1: EHDV-1; Lane 2: EHDV-2; Lane 3: EHDV-318; Lane 4: EHDV-318; Lane 5: EHDV318; Lane 6: EHDV-4

Using SDS-polyacrylamide gel electrophoresis (SDS-PAGE), the EHDV genome also showed 10 bands, which correspond to viral dsRNA genome segments, typical of EHDV serogroup (Fig. 2). However, the cognate genome segments of the Sudanese and the North American serotypes of EHDV migrated to the same length but with different electrophoretic patterns. The dsRNA genome profiles for the Sudanese and the North American EHDV serotypes showed different migration level for segment 2 and 4. Also segment 7, 8 and 9 showed different migration levels.

Segment 9 of the North American EHDV serotype 1 migrated at faster rate compared to cognates of other EHDV serotypes. Segment 10 of the Sudanese EHDV-318 migrated at slower rate compared to cognates of the North American EHDV serotypes 1 and 2.

DISCUSSION

The EHDV isolates used in this study represent a range of different EHDV serotypes collected from different location in North American and Central Africa. The agarose gel electrophoresis could be used to facilitate rapid detection of the 10-dsRNA genome segment of EHDV serogroup. Using SDS-PAGE, the EHDV viral genome showed 10 dsRNA bands, typical of EHDV serogroup. The dsRNA of EHDV serotypes 1, 2, 4 and 318 dsRNA migrated to the same length but with different electrophoretic patterns[2]. The nucleic acid analysis of Sudanese and the North American serotypes of EHDV serogroup by SDS-PAGE showed 10 distinct RNA segments, which represents the characteristic pattern of Orbiviruses. Tentative diagnosis of EHDV infection could be made using SDS-PAGE technique. However, sufficient electrophoretic variation exists in field isolates of EHDV to present different electrophoretic patterns between serotypes. Therefore, different serotypes of EHDV may have the same electropherotype and different Orbiviruses may have indistinguishable electropherotypes[15]. It is well documented that other members of the orbivirus genus including Blue Tongue Virus (BTV), palyam serogroup of orbiviruses and African Horse Sickness Virus (AHSV) have 10 dsRNA genome segments[12,16]. Thus, electrophoretic patterns themselves have no definitive diagnostic value for specific identification of a particular EHDV serotype or even for detection of EHDV serogroup. Using agarose gel, the genomes of Sudanese and the North American EHDV serotypes migrated to the same length despite the different electropherotypic patterns of their dsRNA. This finding suggested that it would be interesting to compare the lengths of EHDV electropherotypes from the Sudan with their North American, Asian and other African counterparts. The difference in length between electropherotypes as determined by their dsRNA migration in PAGE, could then be used as a valuable tool to study the epidemiology of EHDV topotypes from different continents[17]. Very little information is available about the RNA homology or heterology among Sudanese isolates of EHDV serogroup.

In conclusion, the scientific data presented in this communication indicated that the agarose gel electrophoresis could be used to facilitate tentative diagnosis of an orbivirus infection including EHDV. However, SDS-PAGE could be used to detect genetic variations among the same serotypes or between different serotypes of EHDV serogroup as determined by their dsRNA genome profiles.

ACKNOWLEDGMENTS

This study was supported by a grant from the Ministry of Science and Technology, Republic of the Sudan Contract number (MOST-2). We would also like to thanks Professor Elzubair Bashir Taha and Professor Eltayeb Idris for their continuous fainancial support to our Molecular Biology Laboratory.

REFERENCES

1. Borden, E. C., R.E. Shope and F.A. Murphy, 1971. Physicochemical and morphological relationships of some arthropod-borne viruses to bluetongue virus-a new taxonomic group. Physicochemical and serological studies. J. Gen. Virol., 3: 261-271.
2. Fenner, F., H.G. Pereira and J.S. Porterfield, 1974. Family and generic names for virus approved by the international committee on taxonomy of viruses. Intervirology, 3: 193-194.
3. Shope, R.E., Macnamara and L.G. Mangold, 1960. A virus-induced epizootic hemorrhagic disease of the virginia white-tailed deer (*Odocoileus virginianus*). J. Exp Med., 111: 155-170.
4. Aradaib, I.E, M.M. Sawyer and B.I. Osburn, 1994. Experimental epizootic hemorrhagic disease virus infection in calves. J. Vet. Diagn. Invest., 6: 489-492.
5. Aradaib, I.E, G.Y. Akita, J.E. Pearson and B.I. Osburn, 1995. Comparison of polymerase chain reaction and virus isolation for detection of epizootic hemorrhagic disease virus in clinical samples from naturally infected deer. J. Vet. Diagn. Invest., 7: 196-200.

6. Work, T.M, D.A. Jessup and M.M. Sawyer, 1992. Experimental bluetongue and epizootic hemorrhagic disease virus infection in California black-tailed deer. J. Wildl. Dis., 4: 623-628.
7. Aradaib, I.E, K.E. Ibrahim, A.E. Karrar and B.I. Osburn, 1999. A review on the diagnosis of orbivirus infection in Veterinary Medicine. Sudan J. Vet. Sci. Anim. Husb., 38: 1-29.
8. Aradaib, I.E., W.W. Smith, J.S. Cullor and B.I. Osburn, 2003. A multiplex PCR for simultaneous detection and differentiation of North American serotypes of bluetongue and epizootic hemorrhagic disease viruses. Comp. Immunol. Microbiol. Infect. Dis., 26: 77-87.
9. Gorman, E.M., 1992. An overview of orbivirus. Proceeding of the 2nd International Symp. On Bluetongue, African Horse Sickness and Related Orbiviruses, pp: 335-347.
10. Mohammed, M.E.H., 1987. Studies on some Sudanese arboviruses. Ph.D. Thesis, University of Surry, United Kingdom.
11. Mohammed, M.E.H., I.E. Aradaib, M.M. Mukhtar, H.W. Ghalib, H.P. Riemann, A. Oyejide and B.I. Osburn, 1996. Application of molecular biological techniques for detection of epizootic hemorrhagic disease virus (EHDV-318) recovered from a sentinel calf in Central Sudan. Vet. Microbiol., 52: 201-208.
12. Abdalla, M.A., I.E. Aradaib and B.I. Osburn, 2002. Evaluation of RT-PCR detection of Sudanese serotypes of epizootic hemorrhagic dsease virus serogroup. Vet. Arhiv., 72: 311-318.
13. Aradaib, I.E., M.A. Abdalla and A.E. Karrar, 2000. Application of biotechnology in diagnostic veterinary medicine. Proceeding of the 2nd Conference of Assiut University, Eygpt.
14. Aradaib, I.E and N.O.M. Ali, 2004. Current status and future prospects of epizootichemorrhagic disease of deer- A review. Vet. Arhiv., 74: 63-83.
15. Hammami, S., and B.I. Osburn, 1992. Analysis of genetic variation of epizootic hemorrhagic disease virus and bluetongue virus isolates by coelectrophoresis of their double strand RNA. Am. J. Vet. Res., 53: 636-642.
16. Mccoll, K.A. and R.A. Gould, 1991: Detection and characterization of bluetongue virus using the polymerase chain reaction. Virus Res., 21: 19-34.
17. Aradaib, I.E., M.E.H. Mohammed, M.M. Mukhtar, H.W. Ghalib and B.I. Osburn, 1997. Serogrouping and topotyping of Sudanese strains of epizootic hemorrhagic disease virus using polymerase chain reaction. Comp. Immunol. Microbiol. Infect. Dis., 20: 211-218.