YYU Veteriner Fakultesi Dergisi, 2011, 22 (1), 39 - 40
ISSN: 1017-8422; e-ISSN: 1308-3651

ORIGINAL ARTICLE

Possible Epidemiological Role of Dogs in Carrying Infectious Bursal Disease Virus (IBDV) to Poultry

Muhammad Atif ZAHOOR[1] Muhammad ABUBAKAR[2] Saima NAIM[3]
Qaiser Mehmood KHAN[4] Muhammad Javed ARSHED[2]

[1] *University of Agriculture, Department of Veterinary Microbiology, Faisalabad, Pakistan*
[2] *National Veterinary Laboratory, Park Road, Islamabad, Pakistan;*
[3] *University of Agriculture, Department of Chemistry, Faisalabad, Pakistan*
[4] *National Institute for Biotechnology and Genetic Engineering, Biotechnology, Faisalabad, Pakistan*

Received: 25.01.2011 Accepted: 18.02.2011

SUMMARY

The present study described the possible role of dogs in the epidemiology of Infectious Bursal Disease Virus (IBDV) and the viability of its fecal-shed. Bursa samples positive to contain IBDV genome through RT-PCR were fed to two dogs. The fecal samples collected at different time intervals (18 hrs to 54 hrs post-fed) were subjected to RT-PCR. No cytopathic effects (CPEs) were observed when IBDV was attempted to grow in chicken embryo fibroblast cells up to five days. Birds orally fed with IBDV-positive suspension showed no clinical signs and gross lesions up to seven days. Results of the present study indicated that stray dogs fed with IBDV affected bursa excreted the virus from 18h up to 48h but viability of IBDV particles through faeces remained questionable. We suggested that dogs might act as a potential source in transmitting IBDV through infected flesh of dead/ sick birds from one farm to another but the viability of the IBDV may be affected possibly by the gastric physiology of the dog.

Key Words *IBD, Dogs, Carrier, Poultry Birds*

İnfeksiyöz Bursal Hastalık Virusunun (IBDV) Kanatlılara Taşınmasında Köpeklerin Muhtemel Rolü

ÖZET

Bu çalışmada; infeksiyöz bursal hastalık virusunun (IBDV) epidemiyolojisinde, köpek dışkısının, etkenin yayılmasındaki muhtemel rolü araştırıldı. RT-PCR ile IBDV yönünden pozitif oldukları belirlenen bursa fabricius örnekleri 2 adet köpeğe yedirildi. Farklı zamanlarda (18-54 saat) köpeklerden alınan dışkı örnekleri IBDV yönünden RT-PCR ile test edildi. IBDV'u civciv embriyo fibroblast hücre kültürüne ekilerek 5 gün sonra yapılan değerlendirmede, sitopatik efekt oluşmadığı görüldü. Civcivlere IBDV pozitif süspansiyon ağız yoluyla verildi ve 7. günden sonra yapılan değerlendirmede, klinik bulgu ve makroskobik lezyon görülmedi. Bu çalışmadan elde edilen veriler, IBDV ile kontamine Bursa fabricius örneklerini yiyen köpeklerin, 18. saatten başlayarak, 48 saatten daha uzun bir süre etkeni dışkı ile yaydıklarını, ancak dışkıdaki virusun canlılığının tam olarak ortaya konulamadığını gösterdi. Çalışmada sonuç olarak, IBDV içeren hasta veya ölü kanatlı hayvanları yiyen köpeklerin, etkeni çiftlikler arasında potansiyel olarak taşıyabileceği, ancak etkenin muhtemelen köpeklerin midelerinde inaktive olduğu düşünüldü.

Anahtar Kelimeler *IBD, Köpek, Taşıyıcı, Kanatlı Hayvan*

INTRODUCTION

Infectious bursal disease (IBD) is an acute and highly contagious viral disease of young chickens characterized by severe damage to the bursa of Fabricius (BF) that subsequently results into immuno-suppression. Infectious bursal disease virus (IBDV) is a member of the genus *Avibirnavirus* in family *Birnaviridae* (Lukert and Saif, 2003).

Two serotypes have been identified. Serotype-I is pathogenic to chickens and differ markedly in virulence whereas serotype-II isolated both from chickens and turkeys is apathogenic (Rosenberger et al. 1998). Despite the adoption of routine vaccination programs and biosecurity measures, IBD is still a major problem in many countries.

An epidemiologic investigation showed that dogs living in close vicinities to avian facilities might act as one of the potential carrier for IBDV (Pages-mante et al. 2004). In Pakistan, like other developing countries, stray dogs have free access to the majority of commercial poultry farms. Further, the farm-labor routinely offers carcasses of dead/ slaughtered sick birds to these dogs instead of their proper disposal. The present study described the possible role of dogs in the epidemiology of IBDV and the viability of fecal-shed IBDV.

MATERIALS and METHODS

Bursa samples positive to contain IBDV genome through RT-PCR were fed to two stray dogs kept in Animal House Facility, Faculty of Veterinary Science. Both the dogs were male and age was around one year. The dogs were kept

Corresponding author: Muhammad ABUBAKAR
National Veterinary Laboratory, Park Road, Islamabad, Pakistan. e-mail: mabnvl@gmail.com

off-feed for 8 hrs prior to administration. The fecal samples collected at different time intervals (18 hrs to 54 hrs post-fed) were subjected to RT-PCR as described (Smiley and Jackwood 2001).

RNA extraction and RT-PCR: Five gram of fecal sample was homogenized in TNE buffer [Tris HCl (pH 8.0)10 mM, NaCl 100 mM, ethylenediamine tetra acetic acid (EDTA) 1 mM]. The homogenate was twice extracted with chloroform. The aqueous phase was incubated with Proteinase K (1mg/ml) and SDS (1%) for 1 h at 37°C. After incubation, it was treated with Phenol: chloroform: Isoamyl alcohol (25:24:1). After ethanol precipitation, pellet was air-dried and resuspended in a 100 µl volume of 90% Dimethyl sulphoxide (DMSO) (Zahoor et al., 2005). The viral RNA was denatured at 95°C for 5 min. prior to RT-PCR. Both the cDNA synthesis and PCR amplification were performed in a single tube using SuperScript™ One-Step RT-PCR (Invitrogen) (Lee et al., 1994). Primers used in the study have been described (Smiley and Jackwood, 2001). Each reaction tube contained 5 µl of viral RNA, 25 µl of 2 X Reaction mix and 1 µl of RT/ Taq. Nuclease free DEPC water was added to final volume of 50 µl. $MgCl_2$ concentration was kept at 2.2 mM.

The reaction conditions consisted of incubation at 50°C for 30 min. for once and then denaturation at 94°C for 30 sec, annealing at 53°C for 30 sec and extension at 72°C for 1 min. A final extension was given at 72°C for 10 min. The reaction steps were repeated for 35 cycles. A 10 µl f the PCR product was electrophoresed (80 V for 40 minutes) on 1.5% agarose gel. The amplified bands were visualized under UV light at a wavelength of 254 nm with Eagle Eye Gel Documentation System (Stratagene, USA) after ethidium bromide staining (0.5µg/ml). The size of the bands was confirmed with the help of a 100-bp DNA ladder as a molecular size marker (Inivtrogen, Life Technologies).

Chicken embryo fibroblast (CEF) cell culture: The viability of IBDV recovered from the dog's intestinal contents was determined. The feco-positive IBDV samples were homogenized in PBS and centrifuged at 3000 X g. The supernatant was clarified and inoculated to 9-day old embryonated chicken eggs (procured from the University Poultry Farms) through chorio-allantoic membrane route (Smiley et al., 2001) and chicken embryo fibroblast cells (CEFs) for the development of gross lesions and cytopathic effects (CPEs), respectively.

The CEFs cell culture was prepared with 9-day old embryonated eggs in Medium-199 (Sigma, St. Louis, MO) supplemented with 10% fetal calf serum, 1000 U/ml of Benzyl Penicllin G (Sodium) and 1000 µg/ml of Streptomycin sulphate, incubated at 37°C as described (Yamaguchi et al., 2000). The supernatant fluid was also administered orally to fifteen experimental broiler birds of 28 day old to confirm the infectivity of IBDV.

RESULTS

An amplified product of approximately 743 bp was obtained after 35 cycles. The procedure was repeated twice to ensure the reproducibility of the results. No difference was found in the length of amplified fragments.

Primers used in the present study amplified a 743 bp fragment from the nucleotide 701 to 1444 of VP2 gene (Smiley and Jackwood, 2001). These primers are licensed by IDEXX laboratories Inc. (Westbrook, ME USA) for commercial use and have been successfully used (Smiley and Jackwood, 2001).

No gross lesions were observed in chicken embryos up to four passages. No cytopathic effects (CPEs) were observed when IBDV was attempted to grow in chicken embryo fibroblast cells up to five days (Yamaguchi et al., 2000) supporting the argument that faecal shed virus is no more viable. Birds orally fed with IBDV-positive suspension showed no clinical signs and gross lesions up to seven days.

DISCUSSION

Pages-mante et al., (2004) reported the shedding and potential viability of IBDV in the faeces of dogs from 24 to 48 hrs. But results of the present study indicated that stray dogs fed with IBDV affected bursa excreted the virus from 18 hrs up to 48 hrs but viability of IBDV particles through faeces remained questionable. We suggested that dogs might act as a potential source in transmitting IBDV through infected flesh of dead/ sick birds from one farm to another but the viability of the IBDV may be affected possibly by the gastric physiology of the dog. Further studies must be directed to sort out the true status of dogs in transmission of IBDV. Faecal shed-IBDV could also be tested for immunogenicity trials as dog-passaged IBDV inactivated vaccine.

Recently, Park et al., (2010) investigated the role of mice as potential carriers of IBDV, three mice were inoculated with a very virulent strain of IBDV and allowed to have contact with three uninoculated mice. Faeces, intestine and pooled liver and spleen collected from inoculated mice 12 and 24 h post-inoculation were positive for IBDV by reverse transcriptase-polymerase chain reaction (PCR)-nested PCR (RT-PCR–nPCR). IBDV was detected by RT-PCR–nPCR in 3/3 samples of intestine and 2/3 samples of pooled liver and spleen from uninoculated in-contact mice at 24 h after exposure.

ACKNOWLEDGEMENTS

We thank S. E. Sommer and D. J. Jackwood (OARDC, Ohio, USA) for their technical assistance. The present work was funded in part by the Ministry of Science & Technology (MoST), Islamabad, Pakistan.

REFERENCES

Lee LH, Ting IJ, Shien JH, Shieh HK (1994). Single-tube, non-interrupted reverse transcriptase-PCR for the detection of infectious bursal disease virus. *J Clin Microbiol,* 32(5), 1268-1272.

Lukert PD, Saif YM (2003). Infectious bursal disease. In: Diseases of Poultry, 11th edn. Eds Y. M. Saif. Iowa State University Press, Ames, Iowa, USA, pp 161-179.

Pages-Mante A, Torrents D, Meldonado J, Saubi N (2004). Dogs as potential carriers of infectious bursal disease virus. *Avian Pathol,* 33(2), 205-209.

Park MJ, Park JH, Kwon HM (2010). Mice as potential carriers of infectious bursal disease virus in chickens. *Vet J*, 183 (3), 352-354

Rosenberger JK, Saif YM, Jackwood DJ (1998). Infectious bursal disease. In: A laboratory manual for the isolation and identification of avian pathogens. 4th edn. Eds D. E. Swayne, J. R. Glisson, M. W. Jackwood, J. E. Pearson, W. M. Reed. American Association of Avian Pathologists, Kennett Square, PA, pp 215-219.

Smiley JR, Jackwood DJ (2001). Genetic stability of the VP2 hypervariable region of four infectious bursal disease virus isolates after serial passages in specific pathogen free chicken embryos. *Avian Dis,* 45, 1-8.

Yamaguchi T, Setiyono A, Kobayashi M, Takigami S, Fukushi H, Hirai K (2000). Infectious bursal disease live vaccines: changes in the virus population during serial passage in chickens and chicken embryo fibroblasts cells. *Avian Dis,* 44, 284-290

Zahoor MA, Hussain I, Khan QM (2005). Two outbreaks of very virulent infectious bursal disease virus in Pakistan. *Vet Rec*, 157, 813-814.