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RESEARCH ARTICLE

Protection of Chickens against Very Virulent Marek's Disease Virus (MDV) by an Infectious Clone of *Meq*-Null MDV Vaccination

Ning Cui[1§], Yanpeng Li[2§], Shuai Su[1]*, Zhizhong Cui[1], Jiabo Ding[3], Mengjiao Kang[3], Peng Sun[1] and Hongfei Zhu[2]

[1]College of Veterinary Medicine, Shandong Agricultural University, Tai'an, Shandong, 271018, China; [2]Biotechnology research institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, China; [3]China Institute of Veterinary Drug Control, Beijing, 100081, China
*Corresponding author: ssu6307@163.com

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A B S T R A C T

To evaluate the immune-protective effect of GX0101Δmeq bacterial artificial chromosome (BAC) which contains an infectious meq-null Marek's disease virus genome, 1-day-old SPF chickens were reared separately in isolators with positive filtered air. On 1 day of age, chickens were immunized with 20 μg of GX0101Δmeq BAC suspended in PBS, and challenge infection with 500 PFU very virulent rMd5 were performed at day 5 and 12 post-immunization separately. During 90 days after challenge, all chickens were recorded and checked for necropsy. The protective index of the two vaccines used was 80 and 40 for CVI988/Rispens and GX0101Δmeq BAC, respectively, after challenged with the very virulent (vv) virus rMd5 at day 5 post-immunization. When challenged with rMd5 at day 12 post-immunization, the protection index of GX0101Δmeq BAC increased to 67%. Except that GX0101Δmeq BAC can confer protection against vv MDV, a delay in the development of MD could be observed in some chickens vaccinated with GX0101Δmeq BAC. On the other hand, compared with CVI988/Rispens, the rescue of GX0101Δmeq BAC in the body is a prerequisite for access to protection. Therefore, there is a blank period after immunization, which provides a chance for infection with the wild MDV.

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INTRODUCTION

Marek's disease virus (MDV), an alphaherpesvirus, causes Marek's disease (MD), which is a lymphoproliferative disease of chickens resulting in T cell lymphomas and immunosuppression (Calnek, 2001). MDV is transmitted by air, causing high mortality in unvaccinated susceptible chickens (Gimeno *et al*., 1999; Zhang *et al*., 2015). Vaccine is the main way to prevent and control MD. Commercial MD vaccine at present include attenuated type I CVI988/Rispens strain, avirulent type II SB1 strain and type III herpesvirus of turkey (HVT) FC126 strain, of those CVI988/Rispens is most effective and widely used (Islam *et al*., 2013; Lupiani *et al*., 2013; Walkden Brown *et al*., 2013; Liu *et al*., 2015).

Traditional MDV vaccine can control MD effectively, but there existed many inconveniences. MDV is cell-dependent virus, and CEF cells must be used in production of the vaccine, which have to be stored and transported in liquid nitrogen to keep ultralow temperature (Lupiani *et al*., 2013), thus making high production cost and inconvenience of storage, transportation and use. What is worrying most is the possible pollution of virus and bacteria in SPF embryos (Wei *et al*., 2012). HVT vaccine can be lyophilized preserved while its bad protection efficacy and character of ease to be affected by material antibodies have limited its widespread use. What is more serious is that the virulent of MDV is continuously growing, and the present MDV vaccine could not provide adequate immune protective efficacy against super virulent MDV (Witter, 1997). As the acceleration development of molecular biology of MDV, especially in the aspect of gene function research (Jarosinski *et al*., 2005; Brown *et al*., 2006), gene recombination technology has provided a new way to obtain effective vaccine, in which construction of MDV

§These authors contributed equally to this study

pathogenic gene deletion strain and DNA vaccine provided good perspective to get more effective vaccine as much as possible (Tischer *et al*., 2002; Lee *et al*., 2008, 2010, 2012).

MDV Chinese strain GX0101, isolated in 2001, is the first reported recombinant MDV field strain with one reticuloendotheliosis virus (REV) long terminal repeat (LTR) insert (Zhang *et al*., 2005; Su *et al*., 2013). We constructed an infectious BAC clone of GX0101 (Sun *et al*., 2009, 2010), and knocked off meq gene in GX0101 genome using recombination technology to construct a meq deletion strain, GX0101ΔMeq (Li *et al*., 2011). GX0101Δmeq is able to replicate in cell cultures stably, and induce better protective immunity against vv MDV challenge than commercial vaccine CVI988/Rispens without any pathogenicity and oncogenicity in chickens (Su *et al*., 2010; Li *et al*., 2011). In the present study, SPF chickens were vaccinated with DNA of infectious clone GX0101Δmeq with meq gene deleted. It could provide certain immune protection against vv MDV rMd5 and offer a new perspective to develop MDV DNA vaccines.

MATERIALS AND METHODS

Chickens and cells: Specific-pathogen-free (SPF) chickens and chicken embryos for preparation of chicken embryo fibroblast (CEF) cultures were purchased from SPAFAS Co. (Jinan, China; a joint venture with Charles River Laboratory, Wilmington, MA, USA). They were free of avian leukosis virus (ALV), reticuloendotheliosis virus (REV) and chicken infectious anemia virus (CAV).

Viruses and plasmids: Recombinant Md5 virus (rMd5) was generated from cosmids derived from the very virulent (vv) Md5 strain as previously described (Reddy *et al*., 2002). Infectious clone GX0101Δmeq with meq gene deleted and pDS-pHAI-US2 containing BAC backbone as immune control were preserved in our lab (Sun *et al*., 2009; Li *et al*., 2011). CVI988/Rispens were commercial vaccine.

Vaccination experiments: GX0101Δmeq BAC DNA was isolated from E. coli strain DH10B using commercially available kits (Qiagen) according to the standard protocols (Sambrook and Russell, 2001). Seventy five 1-day-old SPF chickens were randomly divided into five equal groups (15 in each group) and reared separately in isolators with positive filtered air. At day 1, each chicken in group 1 was immunized by intramuscular (i.m.) injection with 20ug of GX0101Δmeq DNA, diluted in 200ul of phosphate buffer saline (PBS) (PH7.2). Two thousands PFU commercial vaccine CVI988/Rispens were intra-abdominally (i.a.) into each chicken in group 2. Chickens in group 3 were immunized by i.m. injection with 20ug of plasmid pDS-pHAI-US2 in PBS (200ul), as an immune control group. Chickens in groups 4 and 5 were immunized by i.m. injection with 200ul PBS, as control groups. Five days later, chickens in group 1, 2, 3 and 4 were challenged i.a. with 500 PFU of vv MDV rMd5. During 90 days after challenges, all dead chickens were recorded and necropsied. The tumor-suspected tissues were examined by histo-sections. At the end, all survived chickens were killed and necropsied. Vaccinal immunity to MD was expressed as a protective index (PI) calculated as the percentage of gross MD in non-vaccinated challenged control chickens minus the percentage of gross MD in vaccinated, challenged chickens divided by the percentage of gross MD in non-vaccinated challenged control chickens × 100.

For further evaluation of the protective efficacy of GX0101Δmeq BAC DNA, another experiment was conducted identical to the above experiment except that SPF chickens were challenged with rMd5 on 12 days post immunization.

Statistics analysis: Statistical analysis was performed with the SPSS statistical software package for Windows, version 13.0 (SPSS Inc., Chicago, IL, USA). Differences between groups were examined for statistical significance by a two-tailed Student T-test. $P<0.05$ were considered statistically significant.

RESULTS

GX0101Δmeq virus was reconstituted *in vivo* from GX0101Δmeq BAC DNA: At 1 day of age, chickens were immunized by i.m. injection with GX0101Δmeq DNA. Four days later, six chickens were randomly selected for sterile acquisition anticoagulant for virus isolation and MDV were isolated from four chickens. To confirm the virus, MDV special plaques were examined by immunofluorescence assay (IFA) with monoclonal antibody (mAb) H19 specific for the MDV-unique protein pp38 or mouse anti-Meq polyclonal serum (Cui *et al*., 1991). The virus that expressed pp38 but not Meq was GX0101Δmeq (Fig. 1). The results were further verified by PCR.

Clinical symptoms and autopsy of chickens infected with rMd5 after immunized with GX0101Δmeq DNA: Chickens were challenged with rMd5 5 days post immunization. In the whole trial, seven chickens died in GX0101Δmeq DNA vaccine group with two chickens developed typical MDV tumor nodules; three chickens died in CVI988/Rispens vaccine group with one chicken developed typical MDV tumor nodules; all chickens were died in pDS-pHAI-US2 or PBS vaccine group with four and five chickens developed typical MDV tumor nodules respectively; no chickens died in negative control group. When chickens were challenged with rMd5 12 days post immunization, during the whole trial: three chickens died in GX0101Δmeq DNA vaccine group with two chickens developed typical MDV tumor nodules; one chicken died in CVI988/Rispens vaccine group with typical MDV tumor nodules; fourteen or thirteen chickens died in pDS-pHAI-US2 or PBS vaccine group with four chickens developed typical MDV tumor nodules respectively; no chickens died in negative control group (Fig. 2, Table 1).

Protective efficacy of GX0101Δmeq DNA vaccination against rMd5 in SPF chickens: To evaluate immune protection efficacy of GX0101Δmeq as a vaccine for SPF chickens, vv MDV rMd5 were challenged at different time after immunized with GX0101Δmeq DNA. In the whole trail, challenged control group and pDS-pHAI-US2 plasmid immunization group all showed 100% MDV-

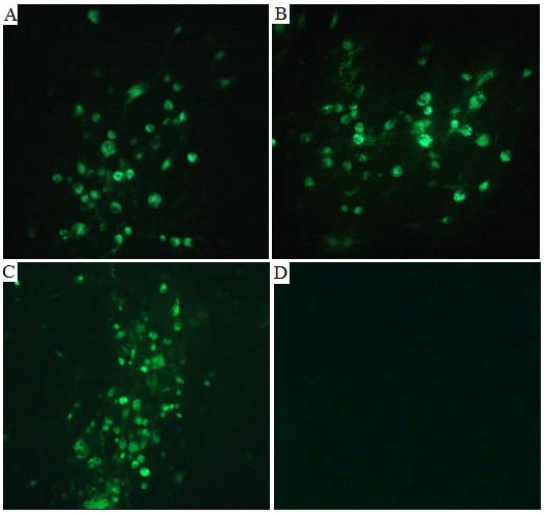

Fig. 1: Immunofluorescence analysis of the reconstituted virus. The mAb H19 specific for the MDV-unique protein pp38, and mouse serum against Meq were used for IFA. Parental virus, GX0101 expressed Meq protein, whereas the deletion mutant virus GX0101ΔMeq did not. The reconstituted virus GX0101ΔMeq was confirmed by staining of MDV-specific pp38 protein. (A) GX0101 with anti-pp38 mAb; (B) reconstituted virus with anti-pp38 mAb; (C) GX0101 with anti-Meq serum; (D) reconstituted virus with anti-Meq serum.

Table 1: Preventive effect of GX0101Δmeq BAC DNA vaccination on tumorigenesis induced by vv MDV

Vaccines	Challenge	Challenge with rMd5 at day 6		Challenge with rMd5 at day 13	
		Died	Tumors	Died	Tumors
GX0101Δmeq	rMd5	7 (47)	2 (13)	3 (20)	2 (13)
CVI988/Rispens	rMd5	3 (20)	1 (7)	1 (7)	1 (7)
pDS-pHAI-US2	rMd5	15 (100)	4 (27)	14 (93)	4 (27)
PBS	rMd5	15 (100)	5 (33)	13 (87)	4 (27)
Control	-	0 (0)	0 (0)	0 (0)	0 (0)

Samples tested in each case were 15. Values in parenthesis indicate percentage.

specific death and pathological symptom; CVI988/ Rispens vaccine group showed 20% and 13% MDV-specific death and pathological symptom respectively in the trial challenged on 5 and 12 days post immunization; GX0101Δmeq DNA vaccine group showed 60% and 33% MDV-specific death and pathological symptom respectively in the trial challenged on 5 and 12 days post immunization; Therefore, based on MD incidence, the protection index of GX0101Δmeq DNA was 40% and 67% in the trial challenged on 5 days and on 12 days post immunization while the protection index of CVI988/Rispens was 80 and 87%, respectively (Table 2).

DISCUSSION

MDV is a double stranded DNA virus, and it can be rescued by transfecting cells with BAC clone containing MDV genome (Schumacher *et al*., 2000). Tischer and others evaluated the immune protective efficacy of attenuated MDV strain 584A p80C infectious clone BAC20 as a vaccine. The results showed that BAC20 can provide certain immune protection as a vaccine even though its immune efficacy (PI: 42%) was lower than that of CVI988/Rispens (Tischer *et al*., 2002). Their researches also compared the protection effect of plasmid DNA in different immune form (dissolved in PBS, calcium phosphate precipitation, chitosan package, in DH10B E. coli and use gene gun, etc) in immunized chickens. The immune effect of plasmid DNA dissolved in PBS was best, and it may mainly because plasmid was in parcel or precipitation state in other forms, which blocked virus being rescued. Petherbridge demonstrated that the immune protection efficacy of CVI988/Rispens infectious clone pCVI988 as a DNA vaccine in 2003 (Petherbridg *et al*., 2003). Results from Tischer and Petherbridge showed that infectious virus was rescued and played its role after chickens were immunized with MDV BAC plasmid, which indicated that MDV BAC DNA induce immune protection efficacy as DNA vaccine in virus form in essence.

Therefore, the most important factor that influenced the immune protection effect of MDV BAC DNA as DNA vaccine was the construction of MDV infectious clone. Currently, except several gene deletion strains reported recent years, there was no better vaccine strain than CVI988/Rispens (Witter *et al*., 2004). Meq gene deletion strain GX0101Δmeq constructed in our lab lost its pathogenicity completely in SPF chickens and could induce better immune protection efficacy than CVI988/Rispens (Su *et al*., 2010; Li *et al*., 2011). Therefore, we speculated that GX0101Δmeq BAC DNA could provide better protection effect than pCVI988. Compared with Petherbridge's experiments, the dose and virulent of the virus used for challenge were different, but both of us have used CVI988/Rispens as control. In Petherbridge's experiments, CVI988/Rispens could provide 100% protection rate, while in the present study, it was only 87%, which demonstrated that the virus we used were more virulent. But even so, the protection efficacy of GX0101Δmeq BAC against MDV challenged on 5 days post immunization was superior to that of pCVI988, which was challenged on seven days post immunization. The results have confirmed our above inference.

Evaluation of immune protection efficacy against challenge on 12 days post immunization was also carried out. The results demonstrated that the immune protection efficacy against challenge on 12 days post GX0101Δmeq BAC immunization were obviously better than challenge on 5 days post immunization. It is mainly because that chicken of six days old was more susceptible to MDV. On the other hand, it takes some time for GX0101Δmeq BAC to rescue enough viruses to exert its immune efficacy in vivo. This showed that compared with CVI988/Rispens; there will be a certain immune phase space for GX0101Δmeq BAC as a vaccine.

The study found that the protection efficacy of GX0101Δmeq-BAC was no better than cell-dependent MDV vaccine CVI988/Rispens. On one hand, it needs some time to rescue enough viruses *in vivo*, and on the other hand, it has a great relationship with the dose of DNA in-taken by body cells. Therefore, further improvement and optimization of immunization dose and pathways for DNA vaccine are necessary. Although the protection efficacy of GX0101Δmeq BAC as a DNA vaccine may not be ideal under laboratory conditions, it might be better under natural infection cases in consideration of the longtime of natural infection and the lower dose than challenge in experiment. But such

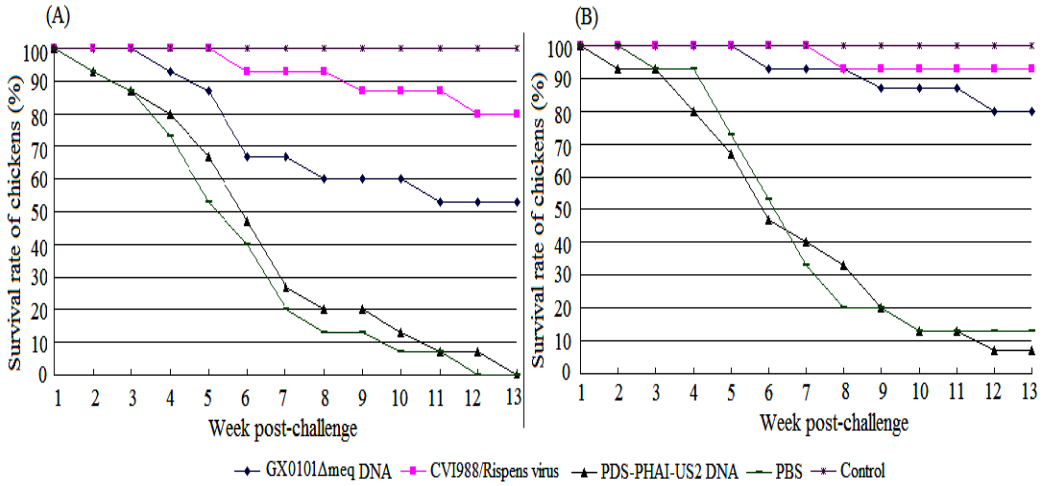

Fig. 2: Survival curves after rMD5-challenge in chickens of each group. (A) Each chicken was immunized at 1 day of age and maintained in isolation for 13 weeks. Non-immunization group served as the negative control. Chickens were challenged with 500 PFU of rMd5 strain at day 6. The mortalities of different groups were recorded weekly. Dead chickens during the experiment were evaluated for MDV-specific gross lesions. (B) Each chicken was immunized at 1 day of age and maintained in isolation for 13 weeks. Non-immunization group served as the negative control. Chickens were challenged with 500 PFU of rMd5 strain at day 13. The mortalities of different groups were recorded weekly. Dead chickens during the experiment were evaluated for MDV-specific gross lesions.

Table 2: Preventive effect of GX0101Δmeq BAC vaccination against vv MDV challenge

Vaccines	Challenge	Challenge with rMd5 at day 6			Challenge with rMd5 at day 13		
		Mortality (%)	MD lesions (%)	PI[α]	Mortality (%)	MD lesions (%)	PI[α]
GX0101Δmeq	rMd5	7 (47)	9 (60)	40[a]	3 (20)	5 (33)	67[b]
CVI988/Rispens	rMd5	3 (20)	3 (20)	80	1 (7)	2 (13)	87
pDS-pHAI-US2	rMd5	15 (100)	15 (100)	-	14 (93)	15 (100)	-
PBS	rMd5	15 (100)	15 (100)	-	13 (87)	15 (100)	-
Control	-	0 (0)	0 (0)	-	0 (0)	0 (0)	-

One-day-old SPF chickens (n=15 in each case) were vaccinated with the GX0101Δmeq BAC or CVI988/Rispens vaccine and challenged 5 or 12 days later with rMd5 strain. Mortality was observed for 13 weeks after chickens challenged with rMd5 strain, and both dead and survival chickens necropsies were subjected to examinations. PI=protection index. [α] indicates significant difference ($P<0.05$) in PI among the two experimental groups.

assumption relies on simulation of natural infection under laboratory conditions. The study also showed that disease time of chickens in GX0101Δmeq BAC immunization group was significantly delayed compared with challenge control group or pDS-pHAI-US2 plasmid immunization control group. Compared with traditional MDV vaccine, BAC DNA is cost saving as it can be prepared in a large-scale by E. coli; it prevents any exogenous pathogen contamination in CEF cells used for production of MDV vaccine; the produced vaccine can be stored under 4ºC or -20□, which is convenient to store and transport, showing a unique advantage. Therefore, BAC DNA can provide a new perspective and attempt for the current and future prevention of MDV (Suter *et al*., 1999).

Conclusions: GX0101Δmeq BAC could not only delay disease time of chicks when infected with very virulent virus, but also provide good immune protection efficacy as DNA vaccine. The rescue of GX0101Δmeq BAC virus *in vivo* is a prerequisite for access to protection.

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REFERENCES

Brown AC, Baigent SJ, Smith LP, Chattoo JP, Petherbridge LJ *et al*., 2006. Interaction of MEQ protein and C-terminal-binding protein is critical for induction of lymphomas by Marek's disease virus. Proc Natl Acad Sci USA, 103: 1687-1692.

Calnek BW, 2001. Pathogenesis of Marek's disease virus infection. Curr Top Microbiol Immunol, 255: 25-55.

Cui ZZ, Lee LF, Liu JL and Kung HJ, 1991. Structural analysis and transcriptional mapping of the Marek's disease virus gene encoding pp38, an antigen associated with transformed cells. J Virol, 65: 6509-6515.

Gimeno IM, Witter RL and Reed WM, 1999. Four distinct neurologic syndromes in Marek's disease: effect of viral strain and pathotype. Avian Dis, 43: 721-737.

Islam T, Walkden Brown SW, Renz KG, Fakhrul Islam AF and Ralapanawe S, 2013. Vaccination-challenge interval markedly influences protection provided by Rispens CVI988 vaccine against very virulent Marek's disease virus challenge. Avian Pathol, 42: 516-526.

Jarosinski KW, Osterrieder N, Nair VK and Schat KA, 2005. Attenuation of Marek's disease virus by deletion of open reading frame RLORF4 but not RLORF5a. J Virol, 79: 11647-11659.

Lee LF, Lupiani B, Silva RF, Kung HJ and Reddy SM, 2008. Recombinant Marek's disease virus (MDV) lacking the Meq oncogene confers protection against challenge with a very virulent plus strain of MDV. Vaccine, 26: 1887-1892.

Lee LF, Kreager KS, Arango J, Paraguassu A, Beckman B *et al*., 2010. Comparative evaluation of vaccine efficacy of recombinant Marek's disease virus vaccine lacking Meq oncogene in commercial chickens. Vaccine, 28: 1294-1299.

Lee LF, Heidari M, Zhang H, Lupiani B, Reddy SM *et al*., 2012. Cell culture attenuation eliminates rMd5ΔMeq-induced bursal and thymic atrophy and renders the mutant virus as an effective and safe vaccine against Marek's disease. Vaccine, 30: 5151-5158.

Li Y, A Sun, S Su, P Zhao, Z Cui and H Zhu, 2011. Deletion of the Meq gene significantly decreases immunosuppression in chickens caused by pathogenic Marek's disease virus. Virol J, 5, 8: 2.

Liu S, Sun W, Chu J, Huang X, Wu Z *et al*., 2015. Construction of recombinant HVT expressing PmpD, and immunological evaluation against *Chlamydia psittaci* and Marek's disease virus. PLoS One, 10: e0124992.

Lupiani B, Lee LF, Kreager KS, Witter RL and Reddy SM, 2013. Insertion of reticuloendotheliosis virus long terminal repeat into the genome of CVI988 strain of Marek's disease virus results in enhanced growth and protection. Avian Dis, 57: 427-31.

Petherbridge L, Howes K, Baigent SJ, Sacco MA, Evans S *et al*., 2003. Replication-competent bacterial artificial chromosomes of Marek's disease virus: novel tools for generation of molecularly defined herpesvirus vaccines. J Virol, 77: 8712-8718.

Reddy SM, Lupiani B, Gimeno IM, Silva RF, Lee LF *et al*., 2002. Rescue of a pathogenic Marek's disease virus with overlapping cosmid DNAs: use of a pp38 mutant to validate the technology for the study of gene function. Proc Natl Acad Sci, USA, 99: 7054-7059.

Sambrook J and Russell DW, 2001. Molecular Cloningcloning: A Laboratory Manualmanual. Third ed., 1. 3 vols. Cold Spring Harbor laboratory Press, Cold Spring Harbor, New York.

Schumacher D, Tischer BK, Fuchs W and Osterrieder N, 2000. Reconstitution of Marek's disease virus serotype 1 (MDV-1) from DNA cloned as a bacterial artificial chromosome and characterization of a glycoprotein B-negative MDV-1 mutant. J Virol, 74: 11088-11098.

Su S, Li Y, Sun A, Zhao P, Ding J *et al*., 2010. Protective immunity of a meq-deleted Marek's disease virus against very virulent virus challenge in chickens. Wei Sheng Wu Xue Bao, 50: 380-386.

Su S, Cui N, Sun A, Li Y, Ding J *et al*., 2013. Sequence analysis of whole genome of a recombinant marek's disease virus strain GX0101 with reticuloendotheliosis virus LTR insert. Arch Virol, 158: 2007-2014.

Sun AJ, Petherbridge LP, Zhao Y, Li YP, Nair VK *et al*., 2009. A BAC clone of MDV strain GX0101 with REV-LTR integration retained its pathogenicity. Chinese Science Bulletin, 54: 2641-2647.

Sun AJ, Xu X, Petherbridge LP, Zhao Y, Nair VK *et al*., 2010. Functional evaluation of the role of reticuloendotheliosis virus long terminal repeat (LTR) integrated into the genome of a field strain of Marek's disease virus. Virology, 397: 270-276.

Suter M, Lew AM, Grob P, Adema GJ, Ackermann M *et al*., 1999. BAC-VAC, a novel generation of (DNA) vaccines: A bacterial artificial chromosome (BAC) containing a replication-competent, packaging-defective virus genome induces protective immunity against herpes simplex virus 1. Proc Natl Acad Sci USA, 96: 12697-12702.

Tischer BK, Schumacher D, Beer M, Beyer J, Teifke JP *et al*., 2002. A DNA vaccine containing an infectious Marek's disease virus genome can confer protection against tumorigenic Marek's disease in chickens. J Gen Virol, 83: 2367-2376.

Walkden Brown SW, Islam A, Islam AF, Burgess SK, Groves PJ *et al*., 2013. Pathotyping of Australian isolates of Marek's disease virus in commercial broiler chickens vaccinated with herpesvirus of turkeys (HVT) or bivalent (HVT/SB1) vaccine and association with viral load in the spleen and feather dander. Aust Vet J, 91:341-350.

Wei K, Sun Z, Zhu S, Guo W, Sheng P *et al*., 2012. Probable congenital transmission of reticuloendotheliosis virus caused by vaccination with contaminated vaccines. PLoS One, 7: e43422.

Witter RL, 1997. Increased virulence of Marek's disease virus field isolates. Avian Dis, 41: 149-163.

Witter RL and Kreager KS, 2004. Serotype 1 viruses modified by backpassage or insertional mutagenesis: approaching the threshold of vaccine efficacy in Marek's disease. Avian Dis, 48: 768-782.

Zhang YP, Li ZJ, Bao KY, Lv HC, Gao YL *et al*., 2015. Pathogenic characteristics of Marek's disease virus field strains prevalent in China and the effectiveness of existing vaccines against them. Vet Microbiol, 177: 62-68.

Zhang Z and Cui Z, 2005. Isolation of recombinant field strains of Marek's disease virus integrated with reticuloendotheliosis virus genome fragments. Sci China C Life Sci, 48: 81-88.