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Research Article

INCIDENCE OF MAREK'S DISEASE IN VACCINATED FLOCKS

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ABSTRACT
A study was undertaken to assess the incidence of Marek's Disease (MD) in vaccinated flocks. Blood, organ and feather follicle samples were collected from fifteen commercial layer farms in which Marek's Disease outbreak was occurred even after vaccination with monovalent and bivalent vaccines. Blood samples were screened for serotype 1 specific132bp repeats and lymphocytes from corresponding positive samples for 132 bp repeats alone used for virus isolation. Organ samples were processed by routine procedure for isolation of virus and treated with antibiotics and filtered (0.45µ) before inoculation. Feather follicles were soaked in SPGA/EDTA buffer and extract used for isolation. 11 days old duck embryos were used to prepare Duck embryo fibroblast culture (DEF). Three passages were carried out in DEF and presence of positive serotype 1 virus was confirmed by PCR for 132bp repeats. Subsequently the isolates were adopted in Chicken Embryo Fibroblast (CEF) culture and produced typical MD plaques. There were three isolates of serotype 1 MD virus recovered from fifteen (20%) field cases. The finding suggests that the Marek's Disease occurrence is prevalent in vaccinated flocks.

Key Words: *Marek's Disease, Serotype 1, Duck Embryo Fibroblast and 132 Repeats*

INTRODUCTION

Marek's disease (MD) is one of the most common lymphoproliferative diseases of chickens which results in mononuclear cell infiltration of one or more of the following tissues: peripheral nerves, gonads, lymphoid organs, iris, muscle, skin and other visceral organs leading to the development of tumours in visceral organs, paralysis, blindness and immune suppression etc., therefore MD has several synonyms like "range paralysis", "neural lymphoma" and "skin leucosis" etc, Witter and Schat, (2003).

Avian Herpes Virus is the causative agent of Marek's Disease and commonly termed Marek's Disease Virus, it belongs to the family Herpers viridae, subfamily alpha herpesvirinae, and genus mardivirus (Marek's disease like viruses). MDV-Herpes virus group has been divided into 3 serotypes based on their biological properties, by using the type specific monoclonal antibodies, Bullow and Biggs, (1975) as serotype 1, 2 and 3. Serotype 1 includes oncogenic MD viruses and the serotype 2 includes non oncogenic MDV and serotype 3 includes Herpes virus of Turkey (HVT), Witter and Schat, (2003). All these serotypes also share common antigens. Therefore, sera against one serotype can interact with the antigens of other 2 serotypes. Serotype 1 MDV strains are further classified into four pathotypes based on induction of lymphoproliferative lesions in vaccinated chickens as mild (m) MDV, virulent (v) MDV, very virulent (vv)MDV, very virulent plus (vv+) MDV, Witter et al., (2005).

Marek's disease (MD) has a tremendous economic impact in the poultry industry, firstly because of cost of vaccination and secondly because of continuing losses due to the disease. The annual losses due to this disease world over have been estimated at more than one billion US dollars. The vaccination can prevent formation of tumor but generation of infectious virus is not prevented. Despite the widespread and successful use of vaccines for the last 40 years, MD virus shows a continuous evolution of virulence. Hence this study has been formulated to assess the occurrence of MD in vaccinated flocks.

MATERIALS AND METHOD

86 blood samples, 54 organ samples and 42 feather follicle samples were collected from fifteen commercial layer farms in which Marek's Disease outbreak occurred even after vaccination. The DNA was extracted from blood by using whole blood DNA extraction kit (Biobasics) and used as temples for PCR. The target gene of PCR was 132 bp repeated sequence of MDV-1 genome, which can distinguish field MDV strain from vaccine strain. The sequences of the primers used for this purpose were R1: 5'-ATG CGA TGA AAG TGC TAT GGA G-3' and R2: 5'-ATC CCT ATG AGA AAG CGC TTG A-3', Tian et al., (2011). Blood samples that contain two or three copies of 132 bp repeats by PCR were used for MDV isolation. The lymphocytes were separated from the positive blood samples by using Ficoll Paques, and were inoculated into primary Duck Embryo Fibroblast (DEF) cells. Organ samples were processed by routine procedure for isolation of virus and treated with antibiotics and filtered (0.45μ) before inoculation. Feather follicles were soaked in SPGA/EDTA buffer and extract used for isolation. DEF was prepared from 11-day-old embryonated duck eggs. Growth medium contained Dulbecos MEM with 5% fetal bovine serum along with 1% 200mM L. Glutamine and pH was adjusted with 7.2% NaHCO3 solution and maintained by adding final concentration of 25mM HEPES buffer. The cells were incubated at 37°C with 5% CO2 for five days for each passage. After three blind passages, the existence of MDV in DEFs was verified by PCR detection of 132 bp repeated sequence. Only the positive samples having no contamination of Avian Leukosis virus (ALV) and Reticuloendotheliosis virus (REV) by PCR (Primers sequences were not shown) were adopted in Chicken Embryo Fibroblast cells.

RESULTS AND DISCUSSION

Three isolates of serotype 1 Marek's Disease Virus were recovered from samples collected from 15 farms (20%). The isolates were confirmed by detection of 132 bp repeats (Fig. 1) by PCR. The isolates were further confirmed by typical plaque formation in Chicken Embryo Fibroblast cells (Fig. 2). Marek's Disease continues to be a most important area of scientific interest, both because of its importance as a major disease affecting poultry health as well as through its status as an excellent model of herpesvirus-induced Tcell lymphomas in their natural avian hosts. As a disease threat, the major concern of the poultry industry is the continuing evolution of virulence and emergence of more virulent MDV pathotypes despite widespread vaccination reported by Biggs and Nair, (2012). The isolation of serotype 1 viruses in the presence of vaccine viruses from flocks where clinical Marek's disease was present suggests that the immunity induced by serotype 2 and/ or 3 vaccines was insufficient and there have been several reports of similar vaccine failures by Zanella, (1982) and Shieh, (1988).

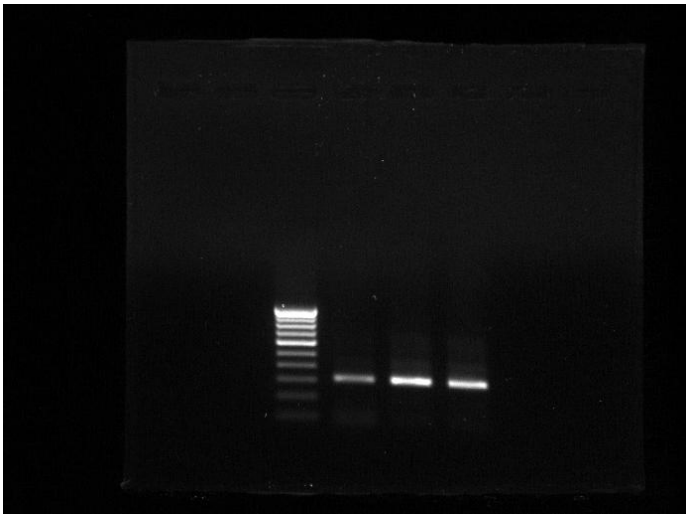

Figure 1: Confirmation of Serotype 1 isolates by 132 repeats in PCR

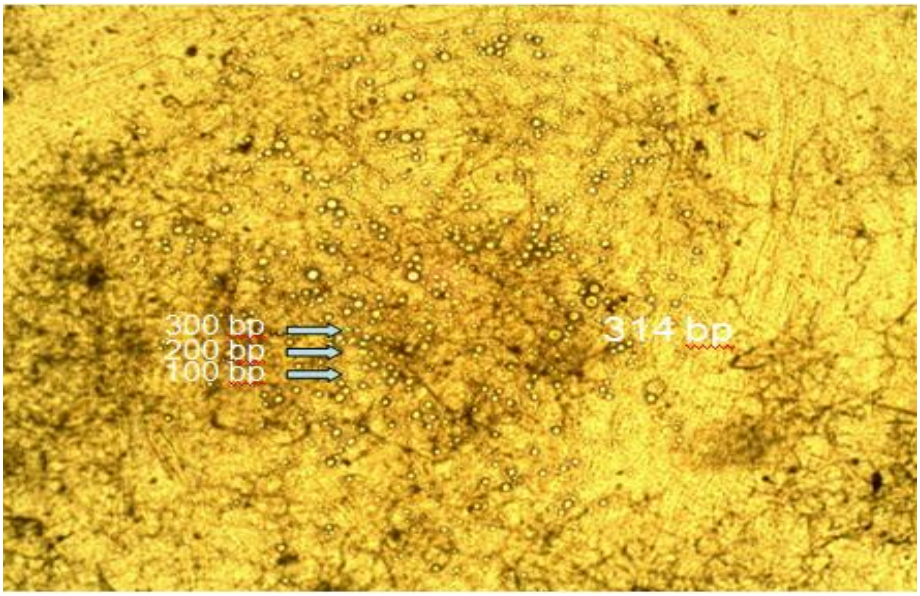

Figure 2: Typical Marek's Disease Viral Plaques in Chicken Embryo Fibroblast Culture (20X)

These failures may have been caused by the selection imposed by the continuous use of vaccines over 40 years, giving rise to variants of altered antigenicity and/or oncogenicity. Based on recent evidence, it is becoming clear that the inability of the existing live attenuated vaccines to prevent replication of the pathogenic strains and induce a ''sterilizing'' immunity is a major factor for driving the virulence. Clearly there is a need for improved vaccines. At the same time, better understanding of the mechanisms of protection induced by the MD vaccines is also important.

Conclusion

There were three isolates of serotype 1 MD virus recovered from fifteen (20%) field cases and the same was confirmed by PCR for 132bp repeats. The finding suggests that the Marek's Disease occurrence is prevalent in vaccinated flocks.

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