ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Protocol

Isolation of Marek's Disease Virus in Tissue Culture and Cytopathological Features in Khartoum State

Rihab M Dafallah[1]* and Sobhi AM Kheir[2]

[1]*Department of Microbiology, Faculty of Science, University of Gezira, Wad Madani, Sudan*

[2]*Central Research Veterinarian, Ministry of Animal Resources, Department of Viral Vaccination, Soba, Khartoum State, Sudan*

***Corresponding Author:** Rihab M Dafallah, Department of Microbiology, Faculty of Science, University of Gezira, Wad Madani, Sudan.

Received: October 09, 2023; **Published:** December 26, 2023

Abstract

Marek's disease (MD) caused by a highly contagious, cell-associated, oncogenic herpesvirus, causes malignant lymphomas in chickens. It has been grown in many types of chicken cell cultures, the isolation of Marek's disease virus (MDV) was performed in chick embryo liver (CEL), chick kidney (CK), and chick embryo fibroblast (CEF) cell cultures and by direct method, we used infected liver as cell culture. The cytopathic effect in different cell cultures was essentially similar. It consisted of grape-like clusters of refractile cells with syncytia. Giemsa-stained cell cultures revealed intranuclear inclusion bodies.

Keywords: *Marek Disease; Herpesvirus; Cell Culture; Khartoum State*

Introduction

Marek's disease is a fatal lymphoproliferative disease in domesticated chickens [1]. The first outbreak of Marek's disease (MD) inception in 1907 by a renowned veterinarian Dr. Joseph Marek at the department of Royal Hungarian Veterinary School in Budapest from which the disease takes its name [2]. Marek's disease virus (MDV) is placed in Order *Herpesvirales*, family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Mardivirus* (Marek's disease-like viruses) and species *Gallid Herpesvirus 2 (GaHV-2)* [3,4].

There are three serotypes belong to the MDV: MDV-1, serotype 1 (Gallid herpesvirus 2), MDV-2, serotype 2 (Gallid herpesvirus 3), and MDV-3, serotype 3 (herpesvirus of turkeys HVT) [3,4]. The birds catch the infected of disease by inhalation the dust from bird's houses [5,6].

The clinical signs recorded on all infected chickens sampled for this study include paralysis of (legs, neck, and wings), gray iris and irregular pupils, depression, loss of weight of some of them, difficulty in breathing, and the skin around feather follicles can be raised and roughened [5-7]. The postmortem confirmed -enlarged sciatic nerves and in tumor formation in sciatic nerves, organs, muscle, and epithelial tissue [8,9].

The morbidity of infected chickens was recorded in both vaccinated and non-vaccinated flocks, due to frequent gene diversity and point mutations of the MDV trains are occur from amino acid substitutions during the virus replication, which reflected in the Meq proteins, the oncoproteins of MDV strains, that might develop MDV virulence [10-12]. MD commonly appears in age between 3 to 4 weeks to a peaks between 12 and 30 weeks [5,6]. Affected birds are susceptible to other infectious diseases due to declining immunity. (14, OIE 2018-2022).

Objective of the Study

The objectives of this study are:

- To isolate the locally circulating strains of MD virus in Sudan.
- To help in choosing the vaccine strains.

Materials and Methods

Collection of samples

Fresh samples were taken from suspected infected chickens (kidney, liver, and spleen with lymphomas were collected and separately processed) and feather follicles where the herpes virus replicates in their muscles.

Autopsy and tissue culture preparation

Infected chickens were cut up with scissors to collect fresh samples and ripped in a mortar and pestle, washed with PBS until they became purpure from blood and centrifuged at 2000 r.p.m for five minutes. The sediment was subsequently resuspended in PBS or cell culture medium containing 10 - 15% calf serum and Dimethylsulphoxide. Antibiotics and mycostatin were added in a concentration of 100 IU/ml of Benzyl Penicillin 100 mg/ml of Streptomycin Sulphate and 50 units of Mycostatin. The suspensions were stored at -80°C and used to inoculate tissue culture monolayers. Otherwise, washed liver tissue suspensions were subjected to trypsinization and processed as direct liver cell cultures (DLC) and observed daily for cytopathic effect (CPE).

Direct infected chicken's liver (DLC) and chicken embryo fibroblast (CEF) cell cultures

The method described by Sharma (1990) was used. CEF cells were prepared from 9 - 10 days old embryos and (DLC) cultures were prepared from infected chicken's liver as a direct method. Cultures were grown in Minimum Essential medium (MEM) in tissue culture flasks or tubes. They were incubated at 37°C in a humidified incubator. Tissue culture on coverslips was prepared in Petri dishes. Enough CO_2 was flown into the incubator at 37°C.

Inoculation of prepared monolayers

About 0.1 - 0.2 ml amount of tissue suspension was used to inoculate CEF monolayers after being washed with PBS. The inoculum was allowed to adsorb for 30 minutes before the maintenance medium was added. Cultures were incubated at 37°C and observed daily for CPE. The Cultures were grown on coverslips, which were fixed in Bouins fixative, Haematoxylin and Eosin were used for staining and examined microscopically. Control cultures were maintained for each cell culture system.

Results

Marek's disease virus was isolated from infected fresh samples (tumor suspension) inoculated onto prepared monolayers CEF cell cultures. Virus isolation was also made from the direct liver cells (DLC) of infected chicken. In control cultures, no cytopathic effect was found (normal growth) (Figure 1). The cytopathic effect found in CEF cells by all isolates was essentially similar and indistinguishable from one another, the cytopathic effect consisted of groups of rounded or grape-like clusters of refractile cells with syncytia (Figure 2). Following some of these rounded cells regress and formed clear spaces on the monolayers. Small, medium, and large plagues developed as early as 4 days post-inoculation (PI). The development of cytopathic effects (CPE) on direct liver cell culture (DLC) from infected chickens rapidly appeared at 3 days and 3 - 5 days PI in cases of inoculation with tumor suspensions, respectively (Figure 3). Stained cultures on coverslips showed intranuclear inclusion bodies, giant cells and syncytia (Figure 4).

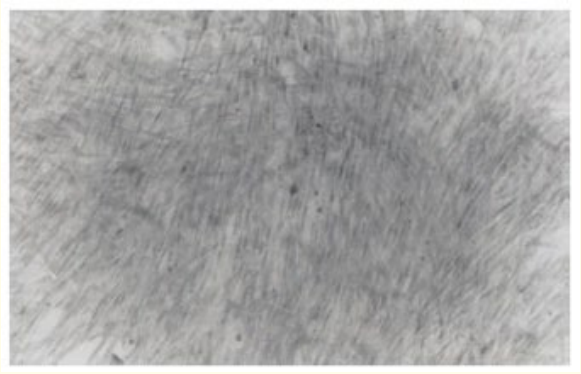

Figure 1: *Section of normal culture (H&E ×10).*

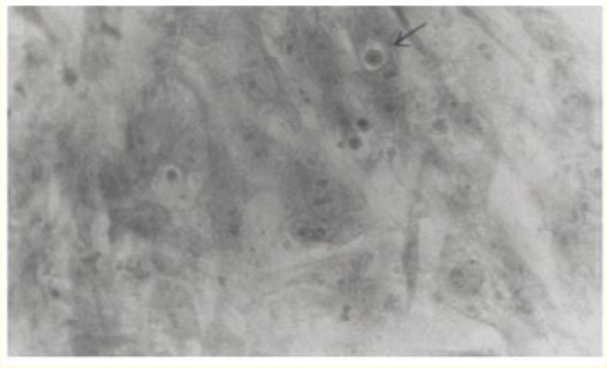

Figure 2: *MD-infected CEL showing grape-like clusters. Wet preparation × 40.*

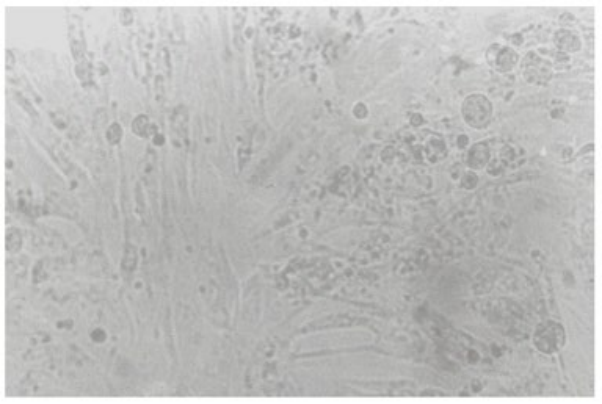

Figure 3: *Infected CEF (wet preparation). Showing rounded refractile cell, syncytia cell.*

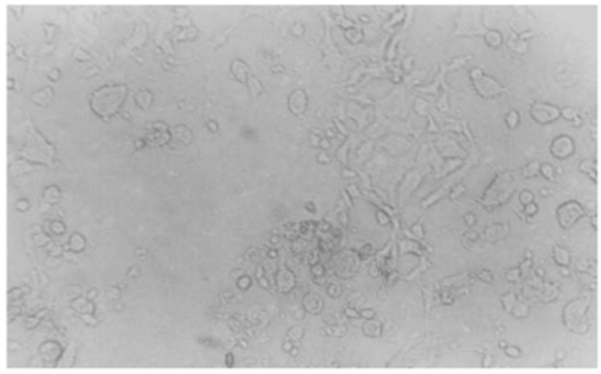

Figure 4: *H&E showed CEF cell cultures showing intranuclear and giant cell inclusion bodies.*

Discussion and Conclusion

Marek's disease virus is a devastating infection worldwide, which causes malignant lymphomas in chickens and causes high morbidity and mortality in chickens [1,2]. Both the clinical signs and postmortem examination reports indicated MDV and it was in agreement with the findings of previous studies in Sudan and other countries [8,15,16]. In this study, CPE have appeared in cell culture as early as three days when freshly processed infected chicken livers were cultured and seen CPE after 3 days. The CPE shape formed by the different isolates in different cell types (DLC, and CEF) were indistinguishable from one another in this study and the previous study also reported the same [8,17]. [17,18] was reported the differences in CPE occur between one laboratory and the other. [19,20] observed CPE in 9-10 days post-inoculation, the different periods in observed CPE may be due to the use of different virus strains, breeds of birds, and other conditions. We found that the inoculated cell cultures produced MD when injected back into chicks [19,20].

Bibliography

1. López-Osorio S., *et al*. "Challenges in the diagnosis and control of Marek's disease virus in Colombia". *Revista MVZ Córdoba* 24 (2019): 7157-7165.

2. Schat KA. "History of the first-generation Marek's disease vaccines: the science and little-known facts". *Avian Diseases* 60 (2016): 715.

3. El-Kenawy AA., *et al*. "Isolation and identification of Marek's disease virus (MDV) from feather follicle epithelium and internal organs of diseased chickens in Dakahlia". *Mansoura Veterinary Medical Journal* 2 (2019): 6-11.

4. Gimeno IM., *et al*. "A Laboratory Manual for the Isolation, Identification and Characterization of Avian Pathogens". Jacksonville, FL: American Association of Avian Pathologists (2016): 249-258.

5. Calnek BW and Witter RL. "Marek's disease". In: Calnek BW, Barnes HJ, Beard CW, McDougald LR, Saif YM, editors. Diseases of poultry. 10th edition. Iowa: Iowa State University Press (1997): 369-413.

6. Gimeno IM. "Stepwise diagnostic approach to investigate a Marek's disease outbreak". In: Pandiri AR, editor. American Association of Avian Pathologist Symposium: An Update on Marek's Disease Vaccination, Diagnosis, and Immunosuppression. Boston, MA: American Association of Avian Pathologists (2015).

7. Abdul-Careem MF., *et al*. "Marek‟s disease virus-induced transient paralysis is associated with cytokine gene expression in the nervous system". *Viral Immunology* 19.2 (2006): 167-176.

8. Awatif and Kheir. "Isolation of MDV in poultry in Tissue Culture and Cytopathological Features". *The Sudan Journal of Veterinary Research* (2001).

9. Witter RL. "Increased virulence of Marek's disease virus field isolates". *Avian Diseases* 41 (1997): 149-163.

10. Shi MY., *et al*. "The emergence of a vv + MDV can break through the protections provided by the current vaccines". *Viruses* 12 (2020): 1048.

11. Sun GR., *et al*. "Chinese variant Marek's disease virus strain with divergence between virulence and vaccine resistance". *Viruses* 9 (2017): 71.

12. Zhuang X., *et al*. "Outbreak of Marek's disease in a vaccinated broiler breeding flock during its peak egg-laying period in China". *BMC Veterinary Research* 11 (2015): 157.

13. Heidari M., *et al*. "Immune responses in cecal tonsils of Marek's disease virus-infected chickens". *Avian Diseases* 59 (2015): 213-226.

14. OIE. "Marek's disease in OIE terrestrial manual". Paris, France: Office International des Epizootics (OIE) (2018).

15. Renz KG., *et al*. "Pathotyping of Australian isolates of Marek's disease virus and association of pathogenicity with meq gene polymorphism". *Avian Pathology* 41 (2012): 161-176.

16. Othman I and Aklilu E. "Marek's disease herpesvirus serotype 1 in broiler breeder and layer chickens in Malaysia". *Veterinary World* 12 (2019): 472-476.

17. Machida Y., *et al*. "Isolation and purification of Gallid herpesvirus 2 strains currently distributed in Japan". *Journal of Veterinary Science* 79 (2017): 115-122.

18. Witter RL. "Increased virulence of Marek's disease virus field isolates". *Avian Diseases* 41 (1997): 149-163.

19. Biggs PM. "Marek's disease in Kaplan. The Herpes viruses". *Academic Press New York* (1973): 557-594.

20. Zhang YP., *et al*. "Sequence analysis of the Meq gene in the predominant Marek's disease virus strains isolated in China during 2006-2008". *Virus Genes* 43 (2011): 353-357.

Volume 19 Issue 10 October 2023