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Case Report

An outbreak of Marek's Disease in a Commercial Poultry Farm in Naththandiya, Sri Lanka: A Case Study

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ABSTRACT

Introduction: Marek's disease (MD) is a major threat to the poultry industry worldwide and significantly affects production and economic stability. In this context, an outbreak in a commercial poultry farm in Naththandiya, Sri Lanka, served as a crucial alarm to avian health management. The clinical signs found during this outbreak not only highlight the virulence of MD virus but also raise questions regarding the effectiveness of the current control measures.

Case report: The outbreak was found in a commercial poultry farm with 4500 Brown Leghorn female chickens including 2500 of 14-weeks-old chickens, and 2000 of 6-weeks-old chickens in Naththandiya, which was characterized by marked mortalities of 15-20 chickens/day in elder chickens and 35-40 chickens/day in 6-weeks-old chickens. The older chickens have been vaccinated with the oral Infectious Bursal Disease (IBD) vaccine at 3 weeks and 11 weeks of age and also with Fowl Pox vaccine at 8 weeks of age. The younger chickens have been given only IBD vaccine at 3 weeks of age. The outbreak showed a wide range of clinical signs, including swollen and closed eyes, nasal discharge, respiratory distress, and anorexia. The post-mortem examination revealed pathognomonic lesions such as grey-white foci of neoplastic tissue in the liver, lungs, and kidneys, enlargement of the sciatic nerve with loss of cross striations, visceral organ enlargement such as liver, kidney, and spleen which indicates the presence of MD virus along with secondary infections caused by *Escherichia coli* (*E. coli*). Histopathological examination and bacterial culture confirmed the MD and *E. coli* infections respectively.

Conclusion: This outbreak highlights the importance of vigilant surveillance, proactive management, and continuous review of vaccination protocols to control the transmission of Marek's disease effectively.

1. Introduction

Marek's disease (MD) is a viral infection that primarily affects chickens but can occasionally impact ducks, geese, turkeys, and quails[1]. Marek's Disease Virus (MDV) is a cell-associated alpha herpesvirus (double-stranded DNA, hexagonal enveloped virus) which is having three serotypes, four pathotypes, and numerous strains with differing degrees of pathogenicity[2]. Three serotypes are as follows, serotype 1; also referred to as Gallid herpesvirus 2. This virus comes under tumor-causing oncoviruses and is linked to virulence or pathogenicity[2]. Serotype 2; the non-oncogenic viruses and Serotype 3; the Herpes Virus Turkey (HVT). Based on its level of virulence, serotype 1 can be categorized into four pathotypes as mild (m), virulent (v), very virulent (vv), and very virulent plus (vv+)[2]. When most people hear the term MD, they typically associate it with fowl paralysis. However, MD can be categorized into various pathological syndromes such as ocular leukosis, cutaneous leukosis, persistent neurological disease, and MD lymphoma[3]. Although they can appear as early as 4 weeks of age, birds with MD typically exhibit clinical symptoms between the ages of 10 and 20 weeks[4]. Therefore, we can distinguish Marek's condition from a very similar condition caused by the avian leucosis virus by finding out the bird's exact age in weeks[5]. Other than that,

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specific post-mortem lesions such as unilateral or bilateral enlargement of peripheral nerves (sciatic, brachial, and vagus) with loss of cross striations, grey-white foci of neoplastic tissue in visceral organs (liver, kidney, spleen, heart, proventriculus, lungs and skeletal muscles) with organomegaly and molecular diagnostics can be also used to identify MD. MD is extremely contagious and spreads horizontally through airborne virus contact, either directly or indirectly[6]. Birds become infected when they inhale virus-containing dust, because contaminated dust may continue to spread the disease for several months[7]. Feathers, feather dander, secretions, and droppings (litter) are common fomites that help to transmit the MD virus rapidly[3]. Infected birds always shed the virus to the surrounding environment as long as they are alive[7]. Therefore, they will spread the virus to the flock by direct or indirect contact between birds and by contamination of the premises through infected molted feathers and dander as the infectious virus is only produced in the feather follicle epithelium[8]. Furthermore, asymptomatic birds also can transmit the virus to others. Darkling beetles may also act as a mechanical vector[3]. Under the natural environment, the cell-free MD virus remains in the contagious stage for 4–8 months at ambient temperature and up to 3 years at 4 ºC (39 ºF)[9]. The organism can be inactivated by cleaning plastic or metal cages of all debris and disinfecting them for 10 minutes with a common disinfectant, such as home bleach diluted one-to-one, quaternary ammonia, organic iodine, or sodium hydroxide[9,10].

This study aims to document and analyze an outbreak of MD in a commercial poultry farm in Naththandiya, Sri Lanka. This case study provides information about the clinical presentation, different diagnostic methods used, pathological discoveries made, and the various challenges faced in managing the disease. Moreover, due to the fact that there are no much-documented cases of MD in Sri Lanka, this study aimed to expand the comprehension of Marek's disease within a poultry environment in the country while supporting the existing pool of knowledge enabling strategies for disease control and increasing awareness about the economic and health consequences associated with MD in similar scenarios.

The neural form associated with MD is known as fowl paralysis or range paralysis. Symptoms of unilateral paresis or paralysis can be observed 3-4 weeks after infection, typically between the ages of 6 to 12 weeks[11]. However, it can be observed in birds that are older than 10 weeks and as young as 3-4 weeks[11]. The splayed leg posture, akin to a bird "doing the splits," involves one leg extended forward and the other backward, creating a distinctive stance[12]. Anorexia, severe depression, dehydration, incoordination of movements, emaciation, and eventual death are other common clinical signs associated with MD-affected birds[13]. During the age of 4-10 weeks, mortality in an infected flock typically increases steadily[9]. Loss of cross striations, unilateral or bilateral enlargement, and grey or yellow discoloration of the sciatic plexus (the Ischiatic nerve) are the characteristic gross symptoms associated with range paralysis or fowl paralysis[11]. Persistent neurological disease is the term for the condition that occasionally results in torticollis or nervous ticks in birds that survive fowl paralysis[14].

Another common pathological form of MD is Marek's disease lymphoma (MD lymphoma). The majority of MD lymphoma cases exhibit minor clinical symptoms such as diarrhea, anorexia, pale hair, and weight loss[15]. The ovary, lung, heart, mesentery, kidney, liver, spleen, adrenal gland, pancreas, proventriculus, intestine, iris, skeletal muscle, and skin are the common tissues that get lesions in MD lymphoma disease[16]. The common lesion is whitish to grayish focal nodular tumors and in histopathological sections, diffuse infiltration of mononuclear cells can be found[17]. The iris becomes pale tan to gray rather than its typical yellow hue when mononuclear infiltrates are discovered within it. It also results in uneven form and a reduction in pupil size[18]. Consequently, ocular lymphoma, or "gray eye," is the term used to describe this illness[12]. In skin lymphoma, the presentation often includes multiple small nodules localized around feather follicles, resulting in a textured, bumpy appearance of the skin[19]. A recent study in Taiwan using 18 MDV-1 strains from 17 vaccinated chicken flocks has revealed through phylogenetic analysis that the distribution of MD lymphoma lesions across organs is known to be influenced by the specific genetic strain of the chicken and the variant strain of the virus[20]. Virulence is characterized by the frequency and intensity of illness that is generated in a vulnerable host. Genetics, maternal antibodies, and immunization status all contribute to the wide variation in MD susceptibility among chickens[21]. The oncogenicity and immune evasion of virulent MDVs (vMDVs) are linked to mutations in genes like *pp38*, *meq*, or *gB*[22]. The more virulent isolates are generally thought to produce more visceral tumors with an earlier onset of tumor mortality and replicate more quickly to gain higher titers *in vivo* than the less virulent isolates[21]. Moreover, the more virulent isolates infect genetically resistant strains of chickens while causing more immune depression, more transient paralysis, and inducing early non-neoplastic death (early mortality syndrome)[21].

2. Case history, clinical observations and laboratory findings

Eight dead 14-week-old Brown Leghorn female chickens and two diseased chickens were presented to the Central Veterinary Investigation Centre (CVIC) by a farmer in the Naththandiya area on the 29th of July 2021, with the following history. Commercial Brown Leghorn chicks have been bought by the farmer from Neil's farm in Naththandiya and a total number of 4500 Brown Leghorn female chickens have been maintained in two flocks. One flock contained 2500 chickens of 14 weeks of age and the other flock contained 2000 chickens of 6 weeks of age. The older batch has been vaccinated with an oral dose of the IBD vaccine at the age of 3 weeks and 11 weeks while the injectable administration of Fowl Pox vaccine at 8 weeks of age. For the younger batch, only an IBD oral dose was given

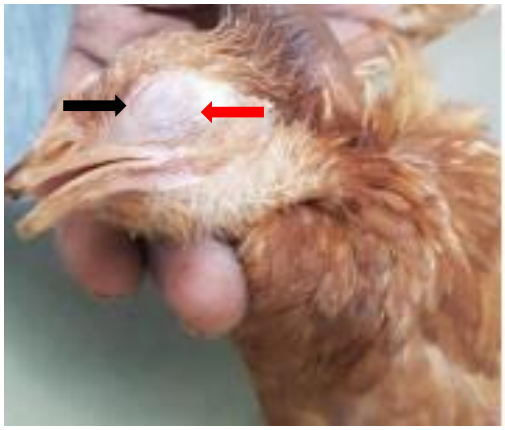

Figure 1. A diseased, live, 14-week-old, Brown Leghorn female chicken with a completely closed (Black arrow) and swollen eye (Red arrow), at the presentation at Central Veterinary Investigation Centre on July 29, 2021.

once at the age of 3 weeks. In the batch of older chickens (14 weeks) 15-20 deaths have been observed per day and in the batch of smaller chickens (6 weeks) 35-40 deaths have been observed per day by the farmer. According to the owner a total of 2000 (approx.) chickens have died within 5 weeks. Infected chickens have been treated with Amoxicillin and Enrofloxacin for one month but haven't got any improvement. Clinical signs observed were, swollen eyes, partial to complete closure of eyes (Figure 1), nasal discharge, wheezing, coughing, anorexia, emaciation, and death.

All the carcasses were subjected to post-mortem examination and samples were collected for histopathology and culture isolation of microorganisms to investigate the cause of death. Post mortem lesions include muscle wasted anemic carcass (Figure 2A), hemoperitoneum (Figure 2B), hepatomegaly with petechial hemorrhages (Figure 2C), extensive yellow foci on the liver (Figure 2D), fibrinous perihepatitis (Figure 2E), Cheesy exudate and paintbrush haemorrhages in the trachea (Figure 2F), splenomegaly (Figure 2G), Yellowish, easily crumbled material on the internal surface of the rib cage and renomegaly (Figure 2H).

Samples from the liver, heart, spleen, kidney, intestines, trachea, and lungs were taken for histopathology as well as culture and isolation of pathogenic microorganisms. Under the histopathological manifestations in the peripheral nerves, neoplastic changes in the cells, inflammatory changes such as diffuse, light-to-moderate infiltration by small lymphocytes along with plasma cells, and edema were observed[23]. Microscopic lesions observed in visceral organs were moderate, multifocal, centrilobular necrosis of the liver; moderate, multifocal tubular necrosis of kidneys; pleomorphic tumor cells infiltration in the renal parenchyma and renal tubular degeneration caused by the pressure of tumor cells. Tumor cells were characterized by large, pleomorphic nuclei with prominent nucleoli. Furthermore, lymphomatous lesions in visceral organs consist of diffusely proliferating small-to-medium lymphocytes, lymphoblasts, and macrophages. The proliferation of reticulocytes and lymphoid cells was observed around the blood vessels in the spleen[24].

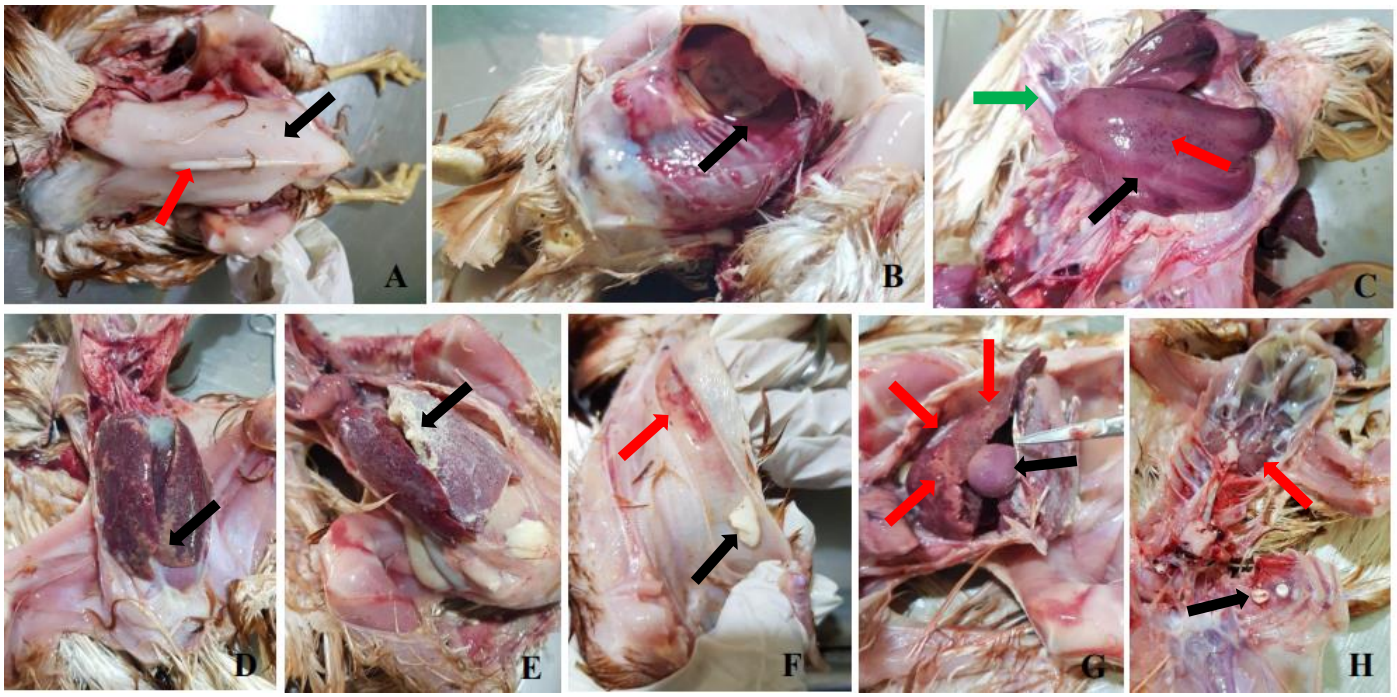

Figure 2. Post-mortem lesions of Brown Leghorn female chickens diseased with Marek disease. **A**: Muscle wasted anemic chicken carcass (black arrow) with prominent keel bone (red arrow) of 14 weeks old Brown Leghorn female chicken on 29th July 2021, **B**: Hemoperitoneum, **C**: Hepatomegaly (black arrow) with petechial hemorrhages (red arrow) and enlargement of the sciatic nerve with loss of cross striations (green arrow), **D**: Extensive yellow foci on the liver, E: Fibrinous perihepatitis, **F**: Cheesy exudate (black arrow) and paintbrush haemorrhages (red arrow) in the trachea, **G**: splenomegaly (black arrow) and whitish nodular tumor like growths on the liver (red arrows), **H**: Yellowish, easily crumbled growths on the internal surface of the rib cage (black arrow) with renomegaly (red arrow).

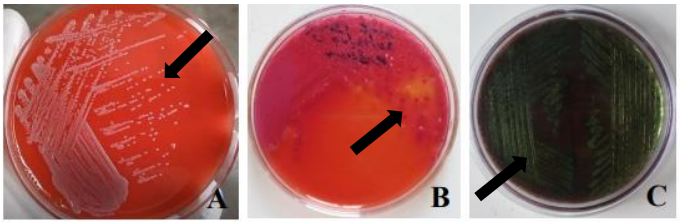

Figure 3. Bacterial culture and isolation results from post-mortem samples taken from 14-week-old Brown Leghorn female chicken carcasses at the CVIC on July 29, 2021. **A:** Suspected *E. coli* colonies in Blood agar plates at bacteriology division center on 30th July 2021, **B:** Suspected *E. coli* colonies in Mac Conkey agar plates at bacteriology division at CVIC on 30th July 2021, **C:** Characteristic green metallic sheen by *E. coli* in EMB agar plates at bacteriology division center on 31st July 2021.

In the lungs, lymphoid growths were in the form of diffuse or sporadic proliferation in the walls of the alveoli and around bronchioles. Based on bacterial culture and isolation results, suspected *E. coli* colonies were detected in Blood agar (Figure 3A) and Mac-Conkey agar plates (Figure 3B) after incubation for 24 hours at 37^{0}C temperature. Suspected colonies were sub-cultured on EMB (Eosin Methylene Blue) Agar plates and incubated at 37^{0}C to

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3. Discussion

According to the history, clinical signs, and gross and histopathological lesions the disease was diagnosed as MD. Culture and isolation results prove that some chickens have been infected with the secondary *E. coli* infection. There is currently no cure for MD, so chickens that have it will keep spreading the virus and can infect other chickens in the flock[18]. PCR, virus isolation, ELISA, qPCR, LAMP, and immunofluorescence are some of the diagnostic techniques that can be used to identify MD[13]. A comprehensive picture and confirmation of Marek's disease can be obtained by histopathological alterations in tissues, obtained from postmortem samples[26]. Due to there is no treatment for MD, prevention is implemented by administering a bivalent vaccine in the egg or for day-old chicks[5] and strictly adhering to biosecurity measures such as cleaning the farmhouses with disinfectants, changing the bedding when acquiring new flocks, restricting visitors, etc[9]. Several types of vaccines are utilized against Marek's disease (MD), either singly or in combination[27]. These include a low pathogenicity strain of serotype 1, a naturally avirulent Turkey Herpesvirus (HVT), and serotype 2 virus. In the context of backyard poultry, the HVT vaccine is frequently administered as a preventive measure[12,27]. Before the introduction of MD vaccines, mortality attributable to Marek's disease (MD) reached up to 60%[11]. Following the development of vaccines, there has been a significant reduction in mortality rates[16]. Research findings indicated that MD vaccines provide over 90% protection, demonstrating their high efficacy and effectiveness[5].

4. Conclusion

The rapid development of clinical signs and high mortality rates within the infected flocks necessitate comprehensive Marek's Disease surveillance and early diagnosis. Post-mortem examinations consistently identified grey-white foci of neoplastic tissue in the liver, lungs, and kidneys; enlargement of the sciatic nerve with loss of cross striations; and visceral organ enlargement such as the liver, kidney, and spleen; and histopathological findings indicated the Marek's disease in the study area. Secondary bacterial infections, notably *E. coli*, were confirmed through bacterial culture, which aggravated the clinical signs and likely contributed to the severity of the outbreak. These findings highlighted the importance of timely vaccination since the affected flock had inadequate or delayed vaccination coverage, which left them more susceptible to Marek's disease and the necessity of strict biosecurity measures. This study not only contributes to the understanding of Marek's disease dynamics in Sri Lanka but also emphasizes the importance of integrated disease management practices in poultry farming in order to prevent future outbreaks. Enhanced awareness and compliance with biosecurity protocols will be crucial to safeguarding commercial poultry operations from this viral disease.

Declarations

Competing interest

There is no conflict of interest.

Authors' contribution

Buddhimali Yashodhara Iluppalla Gamage designed the study, collected the data, and prepared the manuscript for submission.

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Availability of data and materials

All data are ready to send for the readers by requesting via email and any communication platform.

Ethical considerations

Ethical issues (including plagiarism, consent to publish,

misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the author.

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