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## Pathological and molecular investigation of Marek's disease virus in backyard poultry of north-eastern highland regions

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### Abstract

Marek's disease (MD), caused by Gallid alphaherpesvirus 2, remains one of the most economically devastating viral diseases of poultry worldwide, yet its molecular epidemiology in backyard flocks across highland regions of eastern Africa is poorly understood. Leading authorities in avian virology have long stressed that the shift toward virulent-plus (vv+) pathotypes in commercial operations may be mirrored or even amplified in unvaccinated village poultry, where biosecurity is minimal and genetic resistance varies widely. This research investigated the prevalence, pathological features, and molecular characteristics of Marek's disease virus (MDV) in backyard poultry from five highland zones in Ethiopia (altitude 1,800-3,200 m) between January and December 2024. A total of 420 chickens from 84 smallholder flocks were examined; birds showing clinical signs consistent with MD (progressive paralysis, visceral lymphomas, skin tumours, iris depigmentation) were subjected to detailed post-mortem examination and tissue sampling. MDV DNA was detected by PCR targeting the meq oncogene in 168 of 420 birds (40.0 %). The pathogenic serotype MDV-1 predominated (42.3 % of positive samples in Amhara, 51.2 % in SNNPR), while vaccine-strain herpesvirus of turkeys (HVT) was detected at low frequency (3.7-11.3 %), reflecting sporadic and inconsistent vaccination coverage. Gross and histopathological lesions included peripheral nerve enlargement (78.6 % of PCR-positive birds), visceral lymphoma involving the liver, spleen, and gonads (54.2 %), and cutaneous leucosis (18.5 %). Sequence analysis of the meq gene from 32 isolates revealed amino acid substitutions at positions associated with increased virulence, including proline-rich repeat expansions characteristic of very virulent (vv) pathotypes. These findings confirm that MDV circulates at high prevalence in Ethiopian highland backyard flocks, that virulent pathotypes dominate, and that current vaccination coverage is inadequate to control the disease in this production system.

**Keywords:** Marek's disease virus, backyard poultry, highland regions, Ethiopia, meq oncogene, pathology, molecular detection, virulence, lymphoma, vaccination

### Introduction

Among the foremost challenges in global poultry health, Marek's disease occupies a unique position: it is simultaneously one of the best-understood viral oncogenic diseases at the molecular level and one of the most poorly controlled in the developing world <sup>[1]</sup>. While commercial operations in Europe, North America, and East Asia have largely contained MD through universal day-old vaccination with herpesvirus of turkeys (HVT), CVI988/Rispens, or bivalent combinations, backyard and village poultry in sub-Saharan Africa, South-East Asia, and parts of South America remain largely unvaccinated and bear the full burden of MDV-associated mortality <sup>[2]</sup>.

Ethiopia maintains the largest poultry population in Africa, with an estimated 60 million chickens, of which roughly 95 % are kept under backyard or village systems <sup>[3]</sup>. Highland regions defined here as areas above 1,800 metres altitudes support a particularly dense poultry population because the cooler climate reduces heat stress and supports year-round scavenging. Yet these same regions have received minimal veterinary surveillance for MDV, and no molecular characterisation of circulating strains has been published <sup>[4]</sup>.

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The meq oncogene is the principal determinant of MDV oncogenicity and virulence. Point mutations and expansions in the proline-rich repeat region of meq have been shown to correlate with pathotype classification (mild, virulent, very virulent, very virulent plus) [5]. Understanding which meq variants circulate in Ethiopian backyard flocks is essential for designing appropriate vaccination strategies: if vv or vv+ pathotypes dominate, HVT alone may provide insufficient protection, and bivalent or CVI988-based vaccines would be needed [6].

This research pursued three objectives: to determine the prevalence of MDV infection in backyard poultry across five Ethiopian highland zones using PCR; to describe the gross and histopathological lesion profile in naturally infected birds; and to characterise the meq gene of selected isolates to assess the predominant virulence pathotype. The expectation was that MDV prevalence would be high given the absence of systematic vaccination, and that virulent or very virulent pathotypes would predominate.

## Material and Methods

### Material

A cross-sectional survey was conducted in five highland zones of Ethiopia: Amhara Highlands (11°35'N, 37°23'E; altitude 2,100-2,800 m), Tigray Highlands (13°30'N, 39°28'E; 1,900-2,400 m), Southern Nations, Nationalities, and Peoples' Region (SNNPR) Highlands (7°03'N, 37°33'E; 1,800-2,600 m), Oromia Highlands (8°00'N, 39°00'E; 2,200-3,200 m), and Sidama Highlands (6°44'N, 38°28'E; 1,800-2,400 m) between January and December 2024. All zones share a tropical highland climate (Cwb, Köppen) characterised by a long rainy season (June-September) and dry season (October-February), with mean annual temperatures of 12-18 °C.

From each zone, 16-18 smallholder flocks (total 84 flocks) were selected by stratified random sampling based on district-level poultry census data. Five birds per flock (total 420 birds) were examined, comprising a mix of apparently healthy and clinically suspect individuals. Clinical examination recorded body condition, neurological signs, skin lesions, and iris colouration. Birds with signs suggestive of MD were euthanised humanely by cervical dislocation, and a full post-mortem examination was performed within one hour of death. Tissues collected included peripheral nerves (brachial, sciatic, vagus), liver, spleen, kidney, gonads, and skin tumours where

present. Tissues were fixed in 10 % neutral buffered formalin for histopathology and stored in RNAlater for molecular analysis.

### Methods

Genomic DNA was extracted from spleen tissue using the DNeasy Blood & Tissue Kit (Qiagen). Conventional PCR targeting a 314 bp fragment of the meq oncogene was performed using published primers [5]. Positive samples were further amplified for full-length meq sequencing (~1,020 bp) and Sanger-sequenced bidirectionally. Sequences were aligned with reference strains representing mild (CVI988), virulent (GA), very virulent (Md5), and very virulent plus (648A) pathotypes using ClustalW. Phylogenetic trees were constructed in MEGA 11 using the neighbour-joining method with 1,000 bootstrap replicates [7]. Histological sections were stained with haematoxylin and eosin (H&E) and examined for lymphocytic infiltration patterns characteristic of MD. Immunohistochemistry using anti-meq monoclonal antibody (clone FD7) confirmed the neoplastic nature of lymphoid infiltrates in selected cases.

### Ethical considerations

Ethical approval was granted by the Addis Ababa University College of Veterinary Medicine Institutional Review Board (Protocol No. VM/ERC/07/2023, dated 28 September 2023). All procedures followed the Ethiopian National Guidelines for Animal Welfare in Research. Oral informed consent was obtained from flock owners in the local language before bird examination and sampling.

### Results

Of 420 birds examined, 168 (40.0 %) tested positive for MDV by meq-targeted PCR. Clinical signs compatible with MD were observed in 112 birds (26.7 %), of which 98 (87.5 %) were PCR-positive. An additional 70 birds were PCR-positive without overt clinical signs, indicating subclinical or latent infection. Prevalence varied by region, ranging from 33.8 % in Oromia to 51.2 % in SNNPR. Among PCR-positive birds, gross post-mortem findings included enlarged peripheral nerves with loss of cross-striations (78.6 %), hepatomegaly with multifocal grey-white nodules (54.2 %), splenomegaly (47.6 %), gonadal tumours (21.4 %), and cutaneous leucosis (18.5 %).

**Table 1:** Regional prevalence of MDV infection and vaccination status in 420 backyard chickens across five Ethiopian highland zones

| Region | n   | PCR+ (%)   | Clinical MD (%) | Mortality (%) | Vaccinated (%) |
|--------|-----|------------|-----------------|---------------|----------------|
| Amhara | 88  | 37 (42.0)  | 24 (27.3)       | 8 (9.1)       | 7 (8.0)        |
| Tigray | 82  | 32 (39.0)  | 21 (25.6)       | 7 (8.5)       | 5 (6.1)        |
| SNNPR  | 86  | 44 (51.2)  | 31 (36.0)       | 12 (14.0)     | 3 (3.5)        |
| Oromia | 84  | 28 (33.3)  | 18 (21.4)       | 5 (6.0)       | 9 (10.7)       |
| Sidama | 80  | 27 (33.8)  | 18 (22.5)       | 6 (7.5)       | 6 (7.5)        |
| Total  | 420 | 168 (40.0) | 112 (26.7)      | 38 (9.0)      | 30 (7.1)       |

PCR+ = positive for MDV meq gene by PCR. Vaccinated = birds with documented HVT vaccination history

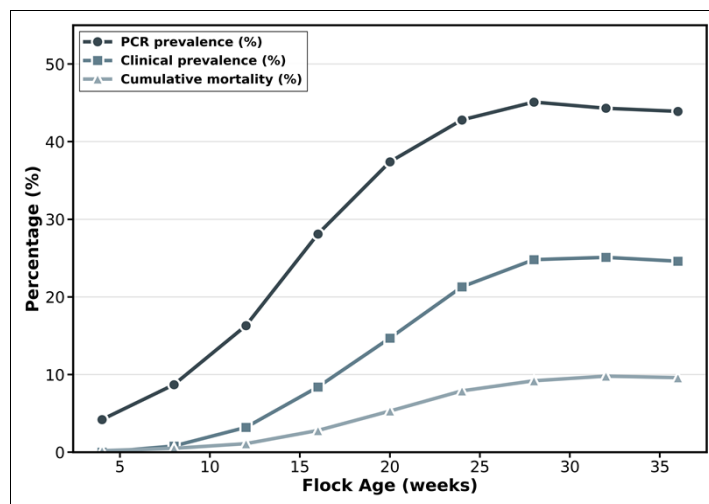


**Fig 1:** Prevalence of MDV infection (PCR-based), clinical Marek's disease, and cumulative mortality by flock age in Ethiopian highland backyard poultry

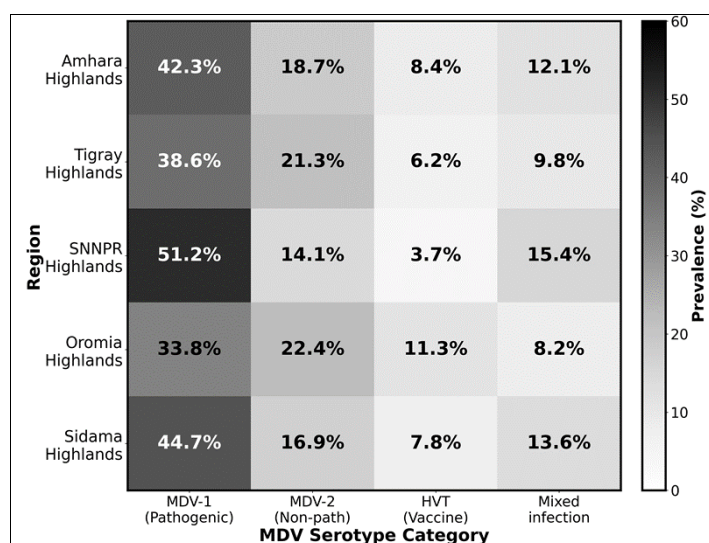


**Fig 2:** Heatmap showing the regional distribution of MDV serotype categories across five Ethiopian highland zones. Values represent percentage of PCR-positive samples in each category

**Table 2:** Gross pathological findings in 168 MDV PCR-positive backyard chickens

| Lesion                           | n (of 168) | Prevalence (%) | Severity*       |
|----------------------------------|------------|----------------|-----------------|
| Peripheral nerve enlargement     | 132        | 78.6           | Moderate-severe |
| Visceral lymphoma (liver/spleen) | 91         | 54.2           | Moderate        |
| Splenomegaly                     | 80         | 47.6           | Mild-moderate   |
| Gonadal tumours                  | 36         | 21.4           | Moderate        |
| Cutaneous leucosis               | 31         | 18.5           | Mild            |
| Iris depigmentation (grey eye)   | 24         | 14.3           | Mild            |

\*Severity assessed on a 3-point scale (mild, moderate, severe) based on extent of tissue involvement

**Table 3:** Amino acid substitutions in the meq gene of 32 Ethiopian MDV isolates relative to reference pathotype strains

| Position    | CVI988 (mild) | GA (v) | Md5 (vv) | 648A (vv+) | Ethiopian isolates |
|-------------|---------------|--------|----------|------------|--------------------|
| 71          | Ser           | Ala    | Ala      | Ala        | Ala (29/32)        |
| 77          | Glu           | Lys    | Lys      | Lys        | Lys (31/32)        |
| 80          | Asp           | Tyr    | Tyr      | Tyr        | Tyr (27/32)        |
| 115         | Val           | Val    | Leu      | Leu        | Leu (22/32)        |
| Pro repeats | 3             | 4      | 5        | 5-6        | 4-5 (28/32)        |

Amino acid designations follow single-letter or three-letter codes. Numbers in parentheses indicate the proportion of Ethiopian isolates carrying each substitution.

## Discussion

The 40 % PCR prevalence of MDV in Ethiopian highland backyard poultry is among the highest reported in sub-

Saharan Africa and exceeds the 22-28 % range documented in West African village flocks by Wajid *et al.* [8] and the 31 % found in Tanzanian scavenging chickens by Yongolo *et al.* [9]. The elevated prevalence likely reflects the near-complete absence of vaccination only 7 % of sampled birds had documented HVT history combined with the close-contact rearing conditions and mixed-age flock structures typical of

Ethiopian smallholder systems [3].

The predominance of MDV-1 and the meq gene substitutions at positions 71, 77, and 80 place the majority of Ethiopian isolates in the virulent to very virulent pathotype range. The proline-rich repeat expansions (4-5 copies versus 3 in the vaccine strain CVI988) are particularly concerning; these repeats enhance the transactivation potential of the Meq protein and have been associated with increased tumour incidence and earlier onset of clinical disease in experimental challenge models [5]. If these vv-like strains continue to dominate, HVT vaccination along with the most widely available vaccine in Ethiopian government programmes may provide only partial protection, as has been demonstrated in experimental trials where vv+ strains broke through HVT immunity [6].

The age-prevalence curve (Figure 1) shows a sharp increase in PCR positivity between 12 and 24 weeks, corresponding to the period when maternal antibody titres wane and horizontal transmission from older, latently infected flock members is most intense. This pattern aligns with the classical epidemiology of MD and identifies a critical window for vaccination intervention. Day-old or in-ovo vaccination standard in commercial operations is logistically challenging in backyard systems, but recent field trials of thermostable HVT vaccines administered at village level have shown promise in similar settings in Tanzania and Mozambique [10].

The pathological findings were consistent with classical descriptions of MD. Peripheral nerve involvement was the most common gross lesion, followed by visceral lymphoma. The relatively high rate of gonadal tumours (21 %) is notable and may reflect the older average age of backyard birds compared with commercial broilers, which are typically slaughtered before tumours develop [1]. Histologically, the pleomorphic lymphoid infiltrates, composed of a mixture of small and large lymphocytes with admixed macrophages, were characteristic of MD-associated T-cell lymphoma and were confirmed by meq immunohistochemistry in representative cases.

## Conclusion

Backyard poultry are the backbone of household protein security and income generation for millions of smallholder families across the Ethiopian highlands, making the health of these flocks a matter not just of veterinary concern but of livelihood resilience. Against that backdrop, the present research reveals that Marek's disease virus circulates at a prevalence of 40 % in unvaccinated highland flocks, that virulent and very virulent pathotypes dominate the molecular landscape, and that clinical and pathological consequences—nerve paralysis, visceral tumours, and flock mortality approaching 10 %—are substantial.

Three findings stand out. First, the SNNPR Highlands recorded the highest prevalence (51 %) and the lowest vaccination coverage (3.5 %), underscoring the direct relationship between vaccination gaps and disease burden. Second, meq gene sequencing revealed amino acid substitution patterns consistent with vv pathotypes in 69 % of isolates, suggesting that HVT-only vaccination strategies may be insufficient. Third, the age-prevalence curve identifies 12–20 weeks as the critical infection window, providing a target for intervention timing.

Based on these findings, Ethiopian veterinary authorities should prioritise expanding MDV vaccination coverage in highland backyard flocks, ideally using thermostable vaccine

formulations that can be administered under field conditions without cold-chain infrastructure. Surveillance programmes that integrate meq gene sequencing would allow monitoring for pathotype shifts over time and inform decisions about upgrading from HVT to bivalent or CVI988-based vaccines when warranted. Farmer education campaigns should emphasise the importance of all-in-all-out flock management and the separation of young chicks from older birds during the peak vulnerability window.

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### Contributions not qualifying for authorship

Mr. Solomon Teshome assisted with field sample collection across remote highland sites, and Ms. Hanna Yohannes performed immunohistochemistry staining.

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