Marek's Disease Vaccination Opened the Door to Rear Indigenous Chickens of Ethiopia Under Confined Management

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ABSTRACT
The extent and effectiveness of Marek's disease (MD) vaccination in reducing the incidence of mortality to natural MD challenge among 3 indigenous chicken ecotypes and Fayoumi chicken that were reared under confined management were investigated from July 2005 to January 2006 at the Debre Zeit Agricultural Research Center in central Ethiopia.

The study was conducted on 546 indigenous chickens (234 Jarso, 90 Tepi, and 222 Horro) and 96 Fayoumi that were vaccinated against MD subcutaneously at neck region (1 mL bid). An equal number of chickens from each ecotype was kept without MD vaccination as a control. Data from daily records of mortality and survival of the chickens were analyzed. Marek's disease vaccination significantly \((P < 0.000)\) increased the survival rate of Jarso and Horro from 36.3% to 82.9% and from 51.4% to 79.3%, respectively. The Tepi ecotype showed improvement in survival rate due to MD vaccination but improvement was not significant. Survival of Fayoumi chickens also increased significantly \((P < 0.05)\) from 94.8% to 100% due to MD vaccination.

Because MD vaccination improved the survival of the indigenous chickens dramatically, it should be considered as an important management strategy while rearing them under confinement. The results of the current study also opened the door and set a baseline for future research efforts on these animals. Further detailed study should be conducted on an immunogenetic basis of variation to respond to MD challenge and vaccination among chicken ecotypes. Study of circulating MD strains isolation in different production systems and vaccine production in the country is recommended.

INTRODUCTION
Indigenous chickens comprise about 99% of the 58 million poultry population in Ethiopia. They constitute by far the largest proportion of the poultry population in Ethiopia and represent a pool of heterogeneous and unimproved individuals that largely differ in production performance, and morphological and genetic characteristics.

Production of indigenous chickens under the scavenging production system is wide-
spread and well-established in the country's villages, even where resource is poor. They are predominantly raised where a traditional family-based free-range scavenging management system is practiced. Thus, the birds are left to depend primarily on what nature offers.2

Attempts have been made at different times to raise indigenous chickens under confined management in different research stations situated in various geographical areas of the country.3-7 However, according to this literature, all attempts have failed due to high morbidities and mortalities of the chickens. These repeated failures have tempted some researchers to conclude that indigenous chickens of Ethiopia are unfit for confined management.6

The disease agent that has been responsible for the high rates of morbidities and mortalities under confinement was found to be Marek's disease (MD). The diagnosis was based on clinical, postmortem, histopathological, and serological examinations7 as recommended by the OIE.8

Marek's disease is a highly contagious and economically important oncogenic or paralytic viral disease of poultry. In chickens it is caused by a highly cell-associated, lymphotropic alphaherpesvirus. Clinical signs include immunosuppression, polyneuritis (paralytic), and T-cell lymphoma formation (carcinogenic) in the visceral and ectoderm-derived tissues.9 The virus matures in the epithelium of feather follicles following infection and sheds from these cells to the environment to infect other birds via inhalation.8,10,11

Marek's disease is controlled essentially by the widespread use of live vaccines administered either in ovo into 18-day-old embryos or into chicks immediately after they hatch.12 It is indicated that MD vaccination reduced the incidence of mortality dramatically from 70% to less than 5%.10,13 This MD vaccination trial is based on the recommendation of Duguma and colleagues7 following MD diagnosis as a severe cause of mortality. In their report, vaccinating indigenous chickens against MD while rearing under confined management would increase survival rate.

Therefore, this study is undertaken to evaluate the extent and effectiveness of MD vaccination in reducing the incidence of morbidity and mortality of indigenous chickens under confined management in central Ethiopia. It also describes the prevalence of mortality in the chicken ecotypes to natural MD challenge in the vaccinated and non-vaccinated indigenous chickens and Fayoumi (reference animal).

MATERIALS AND METHODS

Study Site
The study was conducted at the Debre Zeit Agricultural Research Center (DZARC) located 45 km southeast of Addis Ababa. The site is located at an altitude of 1900 masl. The area receives an average annual rainfall of 851 mm and a minimum and maximum temperature of 8.9°C and 26.2°C, respectively. The average humidity level of the site is 58.6%.

Origin of Study Animals
Eggs of indigenous chickens were purchased from different geographical areas of the country: Horro, Tepi, and Jarso located in the west, southwest, and east of the country, respectively. The chickens were named after their area of origin. The eggs were collected and transported to DZARC for hatching. Fayoumi eggs were collected and hatched from the poultry farm of DZARC.

Management of Study Animals
The hatchery room was cleaned and disinfected with 1% formalin spray 3 hours before the arrival of the eggs. The eggs were selected for physical quality and fumigated for hygiene with 17 g potassium permanganate + 100 mL of 20% formalin and incubated for hatching. Three hours before transfer of eggs from the setter to the hatcher and before each candling, 1% formalin was sprayed in the hatchery room to disinfect and avoid infection of the ovo with
pathogenic MD virus while in the hatchery. Egg candling was undertaken at 7 and 18 days of setting.

All chickens were vaccinated against Newcastle disease with HB1 at Day 1 and LaSota at Days 21 and 56 and every 6 months of life in accordance with the producer's recommendation. Marek's disease vaccine (1 mL) was given on the neck subcutaneously to 96 Fayoumi, 222 Horro, 90 Tepi, and 234 Jarso chickens; an equal number of chickens from each type received the same management except lack of MD vaccination to serve as a control.

The poultry house (both brooder and grower houses) with all poultry equipments and beddings were disinfected by 2% formalin 1 day before the introduction of the chickens. The house was bedded with Teff straw and had infrared bulbs for heating. The baby chicks were supplied with starter ration and clean potable water. The chicks were fed a commercial starter ration during the brooding period (starter phase), which lasted for 2 months. After the end of the starter phase, the chickens were transferred to a grower house where they were fed a grower ration for about 3 months. Antibiotics and vitamins were supplied for all chick flocks under study when disease was suspected in a pen.

**Study Design and Data Collection**

The study involved 4 chicken types (3 indigenous ecotypes and Fayoumi) that were broadly grouped into 2 treatment regimes: MD vaccinated and MD non-vaccinated. Data on mortality parameter were collected daily from chickens until 21 weeks of age.

**Data Analysis**

Mortality prevalence was calculated by dividing the number of animals who died due to MD signs by the total number of animals examined. Percentages (%) were used to measure mortality rate. The susceptibility and resistance rate to natural MD challenge was measured by mortality rate. The response of the chickens to MD vaccination was measured by improvement in mortality rate. Measurement of association between mortality prevalence and chicken genotypes, chicken genotype susceptibility/resistance to natural MD challenge, and vaccination trial was tested using chi-square ($\chi^2$). Thus, the data were analyzed using simple descriptive statistics and a chi-square test at 95% confidence interval ($\alpha = 5\%$). The Version 12 SPSS software (SPSS Inc., Chicago, Ill, USA) was employed for data analysis.

**RESULTS**

**Total Prevalence of Mortality**

The prevalence of mortality in the 4 chicken genotypes (MD vaccinated and non-vaccinated) are presented in Table 1. The level of susceptibility and resistance rate to MD natural challenge varied between chicken genotypes (Table 2). The response of the 4 chicken genotypes to MD vaccination varied between ecotypes (Table 3). Mortality

<table>
<thead>
<tr>
<th>Breed</th>
<th>MD Vaccine</th>
<th>Survival</th>
<th>Death</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fayoumi</td>
<td>Non-vaccinated</td>
<td>N</td>
<td>91</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>94.8</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>Vaccinated</td>
<td>N</td>
<td>96</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>100.0</td>
<td>0</td>
</tr>
<tr>
<td>Horro</td>
<td>Non-vaccinated</td>
<td>N</td>
<td>114</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>51.4</td>
<td>48.6</td>
</tr>
<tr>
<td></td>
<td>Vaccinated</td>
<td>N</td>
<td>176</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>79.3</td>
<td>20.7</td>
</tr>
<tr>
<td>Tepi</td>
<td>Non-vaccinated</td>
<td>N</td>
<td>56</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>62.2</td>
<td>37.8</td>
</tr>
<tr>
<td></td>
<td>Vaccinated</td>
<td>N</td>
<td>62</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>68.9</td>
<td>31.1</td>
</tr>
<tr>
<td>Jarso</td>
<td>Non-vaccinated</td>
<td>N</td>
<td>85</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>36.3</td>
<td>63.7</td>
</tr>
<tr>
<td></td>
<td>Vaccinated</td>
<td>N</td>
<td>194</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>82.9</td>
<td>17.1</td>
</tr>
</tbody>
</table>
variation was also observed within chicken genotype due to presence or absence of MD vaccination and, simultaneously, the impact of MD vaccination to uplift survival rate through reducing mortality was measured and noted (Table 4).

**Table 2. Response of Health Performance of Chicken Ecotypes to Natural MD Challenge.**

<table>
<thead>
<tr>
<th></th>
<th>Fayoumi</th>
<th>Horro</th>
<th>Tepi</th>
<th>Jarso</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (%)</td>
<td>94.8</td>
<td>51.4</td>
<td>62.2</td>
<td>36.3</td>
</tr>
<tr>
<td>Death (%)</td>
<td>5.2</td>
<td>48.6</td>
<td>37.8</td>
<td>63.7</td>
</tr>
</tbody>
</table>

χ² significance test
- Fayoumi —
- Horro 0.000* —
- Tepi 0.000* 0.081† —
- Jarso 0.000* 0.001* 0.000* —

*†‡Chi-square statistic was significant at the 0.000, 0.001, and 0.1 level, respectively.

Of the MD non-vaccinated Fayoumi chickens, 94.8% survived and 5.2% died whereas from an equal number of MD vaccinated Fayoumi chickens, 100% survival was observed. Of MD non-vaccinated Horro ecotypes 51.4% survived and 48.6% died while 79.3% survived and 20.7% died from the MD vaccinated group. Of MD non-vaccinated Tepi ecotypes, 62.2% survived and 37.8% died while 68.9% survived and 31.1% died from the MD vaccinated group. Of MD non-vaccinated Jarso ecotypes, 36.3% survived and 63.7% died whereas 82.9% survived and 17.1% died from the MD vaccinated group (Table 1).

**Response to MD Natural Challenge**

The survival rate of MD non-vaccinated Fayoumi chickens differed significantly from indigenous chickens in the presence of natural MD challenge. The response of survival rate of non-vaccinated Tepi, Horro, and Jarso to natural MD challenge also differed significantly from each other. The survival rate of MD non-vaccinated chickens was in the order of Fayoumi, Tepi, Horro, and Jarso, respectively. In other words, the degree of susceptibility to MD challenge as measured by mortality rate differed between chicken genotypes. Fayoumi chickens showed a considerable degree of resistance to MD challenge whereas Jarso was more susceptible to death from MD. Tepi and Horro showed moderate resistance to MD in that the survival rate of Tepi and Horro was significantly higher than Jarso to MD natural challenge. The resistance level of Tepi and Horro ecotypes did not deserve much attention as only 51.4% of Horro and 62.2% Tepi survived until 21 weeks of life (Table 2).

**Response to MD Vaccination**

Marek’s disease vaccination increased the survival rate of Fayoumi breed, differing significantly (P < 0.000) from our indigenous chickens. In the presence of MD vaccination, the survival rate of Jarso and Horro also differed significantly (P < 0.1) from that of Tepi ecotype. Marek’s disease vaccination brought better survival rate of the chickens that ranked in the order of Fayoumi, Jarso, Horro, and Tepi, respectively (Table 3).

From the results of Table 2 and 3, Jarso ecotype seemed to be an ideal indicator and might serve as a standard susceptible check whereas Fayoumi chickens might serve as a standard resistance check to monitor survival in MD-related research activities and challenges.

**Table 3. Response of Health Performance of Chicken Ecotypes to MD Vaccination.**

<table>
<thead>
<tr>
<th></th>
<th>Fayoumi</th>
<th>Horro</th>
<th>Tepi</th>
<th>Jarso</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (%)</td>
<td>100</td>
<td>79.3</td>
<td>68.9</td>
<td>82.9</td>
</tr>
<tr>
<td>Death (%)</td>
<td>0</td>
<td>20.7</td>
<td>31.1</td>
<td>17.1</td>
</tr>
</tbody>
</table>

χ² significance test
- Fayoumi —
- Horro 0.000* —
- Tepi 0.000* 0.051† —
- Jarso 0.000* 0.322 0.006† —

*†‡Chi-square statistic was significant at the 0.000, 0.05, and 0.1 level, respectively.
The survival rate of the chickens improved due to MD vaccination. MD vaccination increased the survival rate of Jarso and Horro significantly ($P < 0.000$) from 36.3% to 82.9% and from 51.4% to 79.3%, respectively. The Tepi ecotype showed improvement in survival rate due to MD vaccination but not significantly. Survival of Fayoumi chickens also increased significantly ($P < 0.05$) from 94.8% to 100% due to MD vaccination (Table 4). Thus, MD vaccination brought a significant change successfully and improved the survival of the chickens dramatically.

Variation was observed within chicken ecotypes to respond to MD vaccination in that Jarso responded strongly to the vaccination while Tepi was weakly. The response of Horro and Fayoumi to MD vaccination was moderate. Therefore, the vaccine-ecotype interaction to reduce MD caused mortality was ranked and more pronounced in Jarso, Horro and Fayoumi chickens than Tepi ecotypes (Table 4).

### DISCUSSION

This study indicated that susceptibility to natural MD challenge as measured by mortality rate varies significantly among the 4 types of chickens. The mortality rate due to natural MD challenge of Fayoumi (94.8%), Tepi (62.2%), Horro (51.4%), and Jarso (36.3%) differed significantly from each other. The research publications on indigenous chickens pertaining to natural MD disease challenge and mortality attack are scarce to compare head to head with these findings. However, the overall mortality of the non-vaccinated chicken groups that were challenged by natural MD was compared crudely with findings of others. The higher mortality rate observed in MD non-vaccinated groups is in agreement with the findings of Blaha and Palya who reported high morbidity and mortality that ranges from 20%-70% in an intensive production system in non-vaccinated chickens. It also agrees with the observation of Lobago and Weldemeskel who recorded mortality as high as 46% in intensively managed non-vaccinated exotic chickens in central Ethiopia. This finding is in agreement with the findings that indigenous chickens of Ethiopia revealed higher susceptibility (very low survival rate) than the exotic breeds, such as Fayoumi, White Leghorn (WLH), and Rhode Island Red (RIR), under the confined management system. The present finding of a high mortality rate agrees with that of Duguma and colleagues who reported mortality rates of 87.5% in Jarso, 60.7% in Konso, 75.0% in Tepi, 58.3% in Tilili, and 67.6% in Horro ecotypes in non-vaccinated indigenous chickens. Unlike the current finding, the birds were cleared in the first 21 weeks of life and failed to survive under the confined management regime. However, the mortality figure is lower in the current finding compared with past findings despite being non-vaccinated ecotypes. This might be due to exposure of the current control groups to infection via inhalation by shedding vaccinal virus as they were placed randomly near each other within 1 dormitory under 1 house. For instance, in this study, the control group of the Tepi ecotype on average was randomly placed unexpectedly within 1 dormitory with 3 of the MD vaccinated other chicken genotypes. Thus, this might result in slight infection that might have led to protection and survival of the control chicken groups in the current study compared with past findings. It has been reported that that the live MD virus can be shed from the vaccinated chicken throughout the lifespan to the environment, infecting other chickens kept nearby via inhalation.
This study indicated that MD vaccination brought a dramatic change through increasing survival rate of chickens from 36.3% to 82.9% in Jarso, 51.4% to 79.3% in Horro, 62.2% to 68.9% in Tepi, and 94.8% to 100% in Fayoumi ecotypes. As research information on indigenous chickens pertaining to MD vaccination trial is scarce, comparison is made with exotic chickens. This finding agrees with a previous report\textsuperscript{15} that observed a mortality rate of 5.51\% of MD vaccinated exotic pullet flocks in Ethiopia. Other studies\textsuperscript{11,13} have indicated that MD vaccination successfully dropped mortality incidence dramatically from ranges as high as 70\% in non-vaccinated to less than 5\% in vaccinated birds. However, the mortality figure of the current finding varies from their reports. It may be attributed to the genetic variation of the birds; dosage and strain of the infecting virus; or variation in management which in turn interacted (host-agent-environment) to determine the outcome of the mortality status in the current study. Blaha\textsuperscript{11} reported that 3 set of factors are related to the disease process, including 1) infective agent factors: viral strain, dosage and route of exposure; 2) host factors: genetic constitution (genotype), age, and sex; and 3) environmental factors: defense-decreasing stressors, such as confining and stocking. These complex set of factors could influence pathogenesis, the incubation period, the age at which an outbreak of MD occur, character and extent of lesions and symptoms, the course of the disease, and the difference in rate and duration of morbidity and mortality in a flock.\textsuperscript{11}

Considerable resistance to MD challenge is observed in Fayoumi chicken. Such resistance of Fayoumi is revealed both in the presence and absence of MD vaccination. The indigenous chickens are generally susceptible to MD but differed significantly. For instance, Jarso seemed more susceptible than others. According to studies conducted so far, indigenous chickens revealed higher susceptibility (very low survival rate) than the exotic breeds, such as Fayoumi, WLH, and RIR, under the confined management system.\textsuperscript{3-7} The reason for higher susceptibility (the poor survival) of our indigenous chickens might be that a very velogenic viral strain is involved and/or our chicken might be genetically predisposed to higher susceptibility. It might also be attributed to immune stressor factors of confining and stocking as they are naturally adapted to a free range scavenging environment and are new to confinement. Thus, as it is shown in this study, without vaccination, our chicken genotypes are very susceptible to MD under confined management. Similarly, variation is observed among the chicken ecotypes in response to the MD vaccination. In this case Jarso seemed to show a dramatic response to MD vaccination while Tepi showed less response to MD vaccination as measured by improvement in survival rate. All chicken genotypes are susceptible to MD virus.

Figure 1. Effect of Marek's disease vaccination on survival of indigenous chicken ecotypes. A. One of Marek's disease (MD) vaccinated group. B. From MD non-vaccinated group.
infection, but they differ greatly in their resistance or susceptibility to clinical MD.\textsuperscript{16} The report showed that several genetic loci are involved to be either resistant or susceptible to MD challenge. Thus, the current study showed that the presence of genetic variation responds to MD challenge and vaccination among chicken ecotypes. In a report by Dessie, considerable genetic variation is present within and between the Jarso, Tepi, Chefe, Tilili, and Horro ecotypes, although the correlation of the genes with MD infection were not included in that study.\textsuperscript{17}

The results of this study show that MD is a serious health problem for indigenous chickens kept under confined management and probably contributed to the collapse of previous attempts. Marek's disease vaccination has dramatically improved the survival rate of indigenous chickens, and hence opened the door for the future research efforts on these animals. Therefore, the need for MD vaccination while rearing indigenous chickens under confined management should be considered as an important management strategy. Further detailed study on immunogenetic basis of variation to respond to the MD challenge and to vaccination among indigenous chicken ecotypes is recommended. As MD is a severe health threat to the poultry industry and yet its vaccine production has not been started in the country, efforts should be made to isolate the strains circulating in different production systems in the country to develop the ultimate MD vaccine.

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REFERENCES