Marek’s disease in local chicken strains of Ethiopia reared under confined management regime in central Ethiopia

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SUMMARY

The prevalence, clinical and pathological manifestations and extent of mortality due to Marek’s disease (MD) was investigated from November 2003 to January 2004 among indigenous chickens of Ethiopia reared under confined management at the Debre Zeit Agricultural Research Center, central Ethiopia. Clinical signs, mortalities, gross and microscopic examinations as well as virological and serological test results were used for the study. Data from 503 local chickens (28 Jarso, 84 Konso, 88 Tepi, 151 Tiliit and 148 Horro) were analyzed. The study indicated an overall significant difference between manifestation of clinical signs in acute (65.2%) and chronic forms (34.8%), respectively (p<0.01). There was also a significant difference among ecotypes in manifestation and forms of MD (p<0.01). The overall mortality was also very high (p<0.01). Mortality assessment among age and chicken ecotypes revealed a significant difference (p<0.01). MD mortality rate was very high in this study where 97.9% diseased birds died. Post mortem investigation revealed nodular tumorous lesions in most visceral organs. Histopathological investigation revealed lymphocyte infiltra
tion of visceral organs and nervous tissues. Serological prevalence study of MD using the Agar gel immunodiffusion test (AGID) technique on 70 randomly sampled local chickens revealed an overall prevalence rate of 72.9%. MD antibody prevalence was not significant among chicken ecotypes. The result of the present study gives a hint for the possible reason behind the report that local chickens are unfit for confined management. MD was a serious health problem, and hence, from this study evidence is emerging to suggest the need for MD vaccination while rearing local chickens under confined management. Further detailed study on MD in local chickens and on the effectiveness of available MD vaccines in reducing the incidence of morbidity and mortality of local chickens under confined management is recommended.

Keywords : Confined management - Local chickens - Marek’s disease - Mortality - Ethiopia.

RÉSUMÉ

La maladie de Marek chez des poulets autochtones Ethiopiens élevés sous un régime confiné en Ethiopie centrale. Par R. DUGUMA, A. YAMI, N. DANA, F. HASSEN et W. ESATU.

La prévalence, les manifestations cliniques et pathologiques et la mortalité liées à la maladie de Marek (MM) chez les poulets éthiopiens élevés en gestion confinée au Centre Agricole de Recherches de Debre Zeit, Ethiopie centrale, ont été étudiées pendant la période allant de novembre 2003 à janvier 2004. Les symptômes, la mortalité, les examens nécropsiques et sérologiques ainsi que les résultats d’essais virologiques et sérologiques ont été mis en oeuvre pour cette étude. Les données obtenues à partir de 503 poulets indigènes (28 Jarso, 84 Konso, 88 Tepi, 151 Tiliit et 148 Horro) ont été analysées. L’étude montre une différence significative entre la manifestation des signes cliniques aigus (65.2%) et chroniques (34.8%), respectivement (p<0.01). Il y a également une différence significative entre les écosyndes sur la manifestation et les formes de la maladie (p<0.01). La mortalité globale était également très importante (p<0.01). L’évaluation de la mortalité en fonction des différents âges et des écosyndes de poulet a permis de mettre en évidence une différence significative (p<0.01). Dans cette étude, 97.9% de poulets malades sont morts de la maladie. L’examen nécropsique a mis en évidence des lésions tumorales dans la plupart des viscères et des tissus nerveux. L’étude sérologique de prévalence par essai d’immuno-diffusion de gel d’agar (AGID) réalisée sur 70 poulets indigènes aléatoirement prélevés a indiqué un taux global de prévalence de 72.9%. La prévalence d’anticorps de MM n’était pas significativement différente en fonction des écosyndes de poulet. Le résultat de cette étude donne une indication pour laquelle les poulets indigènes ne sont pas adaptés à l’élevage confiné. MM est un problème de santé publique. Par conséquent, cette étude montre le besoin de vaccination contre cette pathologie. Il semble désormais important de réaliser une étude sur l’efficacité des vaccins disponibles contre la MM, dans les conditions d’élevage liées à la gestion confinée des animaux.

Mots-clés : Gestion confinée - poulets indigènes - la maladie de Marek - mortalité - Ethiopie.

Introduction

Poverty, malnutrition, ill health with high mortality, unemployment and illiteracy with other poor social services, and hence reliability on foreign aid to sustain the life of ever-increasing human population are the main features of Ethiopia [18]. Intensification of poultry farming for egg and meat production is an efficient way to bridge most of these social gaps by supplying food, income and employment. However, such system of chicken production has the unfortunate consequences that disease outbreak of various etiologies occur more frequently.

Poultry production in Ethiopia can be classified into three categories namely : traditional, small-scale and large-scale commercial farms. The former is based on indigenous chicken types whereas the later two production systems are characterized by more intensively managed exotic chicken lines [3]. Hence, local chicken production in Ethiopia is limited to traditional family-based free-range scavenging management system. The attempts have been made to raise indigenous (local) chickens under confined management in different research stations situated in various geographical areas of the country [16], [1], [5]. However, the repeated attempts have faced serious problem of high morbidities and mortalities. This has led to a tendency to conclude that indigenous chickens of Ethiopia are unfit for confined management [13]. The probable disease agents, which have been suspected for such morbidities and mortalities in confinement, were reported to be coccidiosis, chronic respiratory disease, Marek’s disease and Salmonella pollurum and nutritional deficiencies [15].

Marek’s disease (MD) is a cell-associated, highly contagious, and economically important oncogenic or paralytic viral disease of poultry. It is caused by a herpes virus, which
is distributed worldwide. The virus matures in the epithelium of feather follicles following infection and it sheds from these cells to the environment to infect other birds via inhalation [4], [6]. Study conducted based on clinical sign and pathological lesion on MD in commercial exotic chicken breeds in Ethiopia has revealed high morbidity and mortality (46%) in non-vaccinated birds [9] and about 5% in MD vaccinated chickens [8]. The cause of high morbidity and mortality of native chickens under confined management is not adequately addressed yet. This study describes the prevalence and manifestations of MD in different local chicken strains kept under confined management in central Ethiopia.

Materials and methods

STUDY SITES

The study was conducted at the Debre zeit Agricultural Research Center 45km south of Addis Ababa. The site is located at an altitude of 1900m above sea level. The area receives an average annual rainfall of 851mm 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

Local chickens were purchased from different geographical areas of the country: Tilili, Horro, Tepi, Konso and Jarso located in the northwest, west, southwest, south and east of the country, respectively. The chickens were named after their areas of origin. Local eggs collected from Horro, Konso and Jarso were transported to Debre zeit Agricultural Research Center for hatching. Eggs from Tepi and Tilili were hatched at the Jimma Agricultural College and Andasa Agricultural Research Center, respectively and the chicks were transported to the Debre zeit Agricultural Research center at day-old.

MANAGEMENT OF STUDY ANIMALS

The collected eggs were selected for different quality and fumigated for hygiene with 17g potassium permanganate + 100ml of 20% formalin and incubated for hatching. All chickens were vaccinated against Newcastle disease with (HB1 and LaSota) in accordance with the producer’s recommendation and transferred to clean and disinfected brooder house. The house was bedded with Teff straw and chicks were placed in pens hatched with infrared bulbs. 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 ration for about 3 months. Antibiotics and feed additives (vitamins) were supplied for all chick flocks under study when disease was suspected in a pen.

STUDY DESIGN AND DATA COLLECTION

Data on health parameters were collected from chickens of 14-20 weeks age ranges. The result of this study was based on these age groups. After 20 weeks the birds were culled due to the increased risk of MD infection from these group to the exotic chickens on the farm.

CLINICAL AND NECROPSY EXAMINATION

Daily follow-up was made to the chicken flocks during the study period (November 2003 to January 2004). Clinical observation on disease signs and symptoms observed in sick birds during the study were closely followed, examined and noted. A macroscopic post mortem (necropsy) examination was conducted on dead birds during the study period. The most obvious abnormality or lesion observed on a number of body organs and/or tissues was examined.

HISTOPATHOLOGY

Tissue samples were collected and submitted according to [10] to the Pathology Section of the National Animal Health Research Center in 10 % buffered formalin, dehydrated in alcohol, embedded in paraffin, sectioned into 4-5 µm thick sections and stained with haematoxylin-eosin. The stained samples were examined by light microscope for histological changes.

VIROLOGY AND SEROLOGY

Blood samples were collected and submitted to National Veterinary Institute according to [2]. Viral agent isolation from buffy coat cells of centrifuged blood cell samples was done according to [11] by inoculating 0.2ml of sample suspension into monolayer cultures of chicken kidney cells and incubated at 38.5°C in a humidified incubator containing 5 % CO₂ and areas cytopathic effects (plaques) were examined for 7 days. Similarly, blood samples for serological tests were collected from wing veins of 70 native chickens and the sera submitted to the National Veterinary Institute according to [2]. AGID test was employed on serum samples. The test was undertaken according to [11] using petridishes coated with 1 % Noble agar in phosphate buffered saline containing 8 % sodium chloride by filling adjacent wells with test serum and the center well with standard antigen and kept in an incubator at 37°C for 24 hours. The development of radial zones of precipitation around the test serum denotes the presence of antibody in the serum and hence of MD infection of the chickens.

DATA ANALYSIS

The data were analyzed using simple descriptive statistics and a chi-square test at 95 % confidence interval (\(\alpha\)=5%). The SPSS (SPSS, 1987) software was employed for data analysis.

RESULTS

Symptoms of the disease were first observed in Jarso chicken ecotypes at first week of life. The morbidity, mortality and fatality rates were widely and intensely spread within and among the local chicken genotypes until 14 weeks of age.
The overall morbidity, mortality and fatality rates as well as MD antibody prevalence rates in the local chickens over the study period were 67.9% (340/503) (Table I), 66.2% (Table II), 97.9% (Table III) and 72.9% (Table V), respectively.

**CLINICAL EXAMINATION**

In this study an overall acute (65.2%) and chronic/classical form (34.8%) of clinical manifestation of the disease were recorded (Table I). In this category, the major signs observed in acute form were found dead without any observed clinical signs (31.5%) and severe depression (33.8%). Of the total chickens with the acute form of MD 28.8% (64/222) were observed in Horro chicken ecotypes. However, in paralytic (chronic) form clinical signs with paralysis of different body parts were observed during clinical examination and death occurred within a few days to several weeks after showing clinical signs. Of the 118 chickens with chronic (paralytic) form of the disease 38.1% (45/118) were from the Tilili ecotypes (Table I).

**MORTALITY AND FATALITY**

The overall mortality of local chickens was 66.2% (333/503) in the present study. The highest mortality was observed within the Jarso ecotypes, 87.5% (28/32) and the least within Tilili ecotypes, 58.3% (88/151) (p<0.01). Comparison of mortality among age groups showed highest mortality at 14 weeks age (14.1%=71/503) and 15 weeks age (16.1%=81/503) and then mortality pattern continued at a decreasing rate (Table II).

An overall fatality rate in the present study was high (97.9%) (Table III), indicating that almost all diseased (sick) chickens ultimately died.

**GROSS AND MICROSCOPIC LESIONS**

Gross lesions either in visceral organs and/or peripheral nerves indicative of lymphoid tumors was observed in all 110 chickens autopsied. The lesions in visceral organs took either nodular, diffuse, or mixed forms with varying degree in size of white or grayish coloration with deep, superficial, or mixed depth in parenchyma of the organs. They had firm consistency and smooth surfaces. Of the autopsy examination made on a total of 110 chickens, 71.8% (79/110) showed the acute (visceral) form, 11.8% (13/110) chronic/paralytic form and 16.4% (18/110) mixed form of visceral and paralytic form. The gross lesions were seen in visceral organs including the liver 69.1% (76/110), spleen 64.5% (71/110), lung 59.1 (65/110), heart 49.1% (54/110), etc (Table IV).

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<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>Jarso</th>
<th>Konso</th>
<th>Tepi</th>
<th>Tilili</th>
<th>Horro</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found dead</td>
<td>14(50.0)</td>
<td>11(21.2)</td>
<td>32(47.8)</td>
<td>19(20.7)</td>
<td>31(30.7)</td>
<td>107(31.5)</td>
</tr>
<tr>
<td>Severe depression</td>
<td>11(39.3)</td>
<td>18(34.6)</td>
<td>25(37.3)</td>
<td>28(30.4)</td>
<td>33(32.7)</td>
<td>115(33.8)</td>
</tr>
<tr>
<td><strong>Total acute form</strong></td>
<td><strong>25(89.3)</strong></td>
<td><strong>29(55.8)</strong></td>
<td><strong>57(85.1)</strong></td>
<td><strong>47(51.1)</strong></td>
<td><strong>64(63.4)</strong></td>
<td><strong>222(65.2)</strong></td>
</tr>
<tr>
<td>Leg and wing paralysis</td>
<td>1(3.6)</td>
<td>5(9.6)</td>
<td>2(3.0)</td>
<td>15(16.3)</td>
<td>11(10.9)</td>
<td>34(10.0)</td>
</tr>
<tr>
<td>Twisted and distorted neck</td>
<td>-</td>
<td>1(1.9)</td>
<td>1(1.5)</td>
<td>1(1.1)</td>
<td>4(4.0)</td>
<td>7(2.1)</td>
</tr>
<tr>
<td>Fail to stand and walk with split legs</td>
<td>-</td>
<td>3(5.8)</td>
<td>2(3.0)</td>
<td>2(2.2)</td>
<td>4(4.0)</td>
<td>11(3.2)</td>
</tr>
<tr>
<td>Combination of various paralysis</td>
<td>2(7.1)</td>
<td>14(26.9)</td>
<td>5(7.5)</td>
<td>27(29.3)</td>
<td>18(17.8)</td>
<td>66(19.4)</td>
</tr>
<tr>
<td><strong>Total chronic form</strong></td>
<td><strong>3(10.7)</strong></td>
<td><strong>23(44.2)</strong></td>
<td><strong>10(14.9)</strong></td>
<td><strong>45(49.9)</strong></td>
<td><strong>37(36.6)</strong></td>
<td><strong>118(34.8)</strong></td>
</tr>
<tr>
<td><strong>Grand Total</strong></td>
<td><strong>28</strong></td>
<td><strong>52</strong></td>
<td><strong>67</strong></td>
<td><strong>92</strong></td>
<td><strong>101</strong></td>
<td><strong>340</strong></td>
</tr>
</tbody>
</table>

**TABLE I.** — Effect of ecotype on manifestations and forms of clinical signs in MD infected intensively managed local chicks.
In general, birds with acute form of MD clinical signs had mainly gross lesions in visceral organs. In paralytic form, the involved nerves were pale or grayish in color enlarged in size and lost the characteristic cross-striations when compared to the non-affected nerves.

Microscopic examination was made on brain, peripheral nerves, liver, spleen, bursa, proventriculus, and heart. In brain microgliosis and lymphocytes perivascular cuffing were observed. In peripheral nerves either massive or moderate infiltration of lymphocytes were recorded. In visceral organs diffuse infiltration or aggregates of lymphocytes were noted.

### SEROLOGY AND VIROLOGY

The attempt to isolate the viral agent at the National Veterinary Institute on kidney cell layers of chicken was not successful. MD prevalence (Antibody prevalence) using agar gel immuno-diffusion (AGID) test was very high, 72.9% (Table V).

In general, chickens under intensive management are not vaccinated against MD in most chicken farms of Ethiopia. However, the present study attested that MD is of comparable importance to the devastating Newcastle disease in intensive poultry farms hence deserving serious attention.
The overall picture of this study showed that the extent and forms of clinical signs (Table I), magnitude of mortality (Table II) and the nature of gross lesions (Table IV) among local chicken ecotypes were significantly different (p<0.01). Once the chickens manifested clinical signs, the ultimate fate of such sick chickens was death (Table III). However, Serological results (the level of antibody prevalence to MD infection) showed non-significant difference among the chicken ecotypes (Table V). This probably indicated that despite equal chance of acquiring MD infection, the extent of disease development, i.e. becoming sick and fate of diseased individual chicken, to some extent varied with the ecotype. This may imply the presence of a degree of resistance and/or susceptibility to MD among chicken ecotypes.

Discussion

The indigenous chickens in the present study are severely attacked by high morbidity and mortality. The magnitude of morbidity and mortality is nearly equal indicating MD is highly fatal. The severe susceptibility observed in this study may be the chickens have been exposed to very severe (velogenic) MD strain or the birds are naturally less resistant to the infection challenge. According to [4], the difference in rate and duration of mortality in a flock and the age at which an outbreak of MD occurs are influenced by a number of factors. These are related to either the infective agent (viral strain, dosage and route of exposure), host factors (genetic constitution, age and sex), or environmental factors (defense-decreasing stressors). According to this author the complex set of factors for pathogenesis, and then the incubation period, character and extent of lesions and symptoms, the course of the disease, morbidity and mortality depend mainly on the above three set of factors.

In addition to viral and/or chicken factors for higher susceptibility indicated previously above, confining and stocking of local chickens without MD vaccination might have contributed to the high mortality rate. This is in agreement with the report of [12] and [4] who have indicated high morbidity and mortality (that ranges from 20-70 %) of MD in intensive production system in non-vaccinated chickens. The observation of high mortality rate in this study also agrees with the findings of [9] in that mortality as high as 46 % have been recorded in intensively managed non-vaccinated exotic chickens in central Ethiopia. However, it differs from the report of [8] who have observed a prevalence rate of 9.23 % and mortality rate of 5.51 % of MD in vaccinated exotic pullet flocks. Probably this variation is attributed to the MD vaccination of their study flocks and also genetic variation of the birds. In this line [12] and [4] have indicated that MD vaccination has brought a difference to commercial poultry farms in successfully controlling MD, and hence its mortality incidence has dramatically dropped from ranges as high as 70 % in non-vaccinated to less than 5 % in vaccinated.

The chicken ecotypes were more susceptible to the acute forms of clinical signs and visceral form of gross lesions. These manifestations and forms of clinical signs and gross lesions across chicken ecotypes showed significant difference (p<0.01).

The course of MD was different among chicken ecotypes in such a way that acute form of the disease was observed in 65.2 % during clinical examination and visceral form of MD was 71.8 % during autopsy examination. According to [12] tumor formation in various organs is the characteristics of MD virus sero-type 1. During current study MD attack at 14 and 15 weeks of age was higher than the subsequent age groups. The occurrence of high magnitude of acute form of clinical signs and also visceral forms during autopsy with varying degree of mortality rate among age groups were in agreement with the reports of [9], [6], [11]. According to [17] there was also a report that acute and/or visceral forms of MD, with recent increasing evolution of virulent viral strain, have been frequently observed currently.

Antibody prevalence to MD in this study is very high, 72.9 %. According to [11] AGID tests are employed most commonly to detect antibody and the presence of antibodies in chickens above 4 weeks of age is an indication of MD infection.

The variation in magnitude of signs, lesions and mortality rates among chicken ecotypes despite nearly equal likelihood to MD infection might be related to genetic variation of the chickens. In this line based on PCR study on the DNA, [14] indicated the presence of genetic variation (distance) among these five chicken ecotypes of Ethiopia. The works of many authors have been cited in the report of [7] that chicken genotypes (strains) that were resistant to MD were observed, and hence the use of such chicken genotypes can serve as alternative control strategies for continuing evolution of virulent MD viral strains in the field due to its reliability, long lasting and environmental soundness.

Conclusion

In conclusion, we have presented three observations that suggest the problems and corrective strategies for rearing local chickens under intensive (confined) management. First, MD is diagnosed in heavily attacked local chickens by present mortality. Second, the level of mortality among various chicken strains is different. Third, lesions of malignant neoplasm (tumors) of visceral organs and other body tissues that seems to be MD virus serotype 1, according to [12], have been frequently encountered. In general, this work is in agreement with previous publications from several authors that have implied an association between MD and host mortality, genetic variation among chickens and visceral tumors in poultry. This report together with the works of [9] and [8] indicates the prevalence and importance of MD in a number of poultry farms of Ethiopia. Hence, with increasing establishment of large and small scale poultry farms MD prevalence reports may suggest production of MD vaccine in the country. Besides, this work also gives a point of reference on which to base further investigations to address mortality problems of local chickens under confined management.
Acknowledgement

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Reference