



Sero-Epidemiology of Marek's Disease Virus on Local and Exotic Chickens in Northwest Ethiopia

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ABSTRACT

Marek's disease (MD) is a lymphoproliferative and neuropathic disease of domestic fowl caused by alphaherpesviruses. The current cross-sectional study with a simple random sampling method was undertaken from January 2018 to May 2020. The current study aimed to estimate sero-epidemiology and assess potential risk factors, it is very important to determine MD sero-positivity. Moreover, to measure the association of host and environmental risk factors, the occurrences and spread of MD were identified in local and exotic chickens in Northwest Ethiopia. A total of 768 serum samples from 3 zones were collected and assayed for Marek's Disease Virus (MDV) antibodies using the indirect enzyme-linked immunosorbent assay test. A questionnaire survey was also conducted to gather information on the potential risk factors of MDV sero-positivity, as well as the status of occurrences and spread in the chicken flocks. In the present preliminary study, a high flock and chicken level of MDV was demonstrated, with an overall seroprevalence of 59.11%. The mixed-effect logistic regression analysis of the host potential risk factors showed that the odds of seropositive for MD was significantly higher in local chickens (OR: 1.70, 95% CI: 1.26-2.28) than exotic chickens, higher in chickens getting non-proper feed (OR: 0.26, 95% CI: 0.13-0.54) than getting proper feed, higher in vaccinated chickens (OR: 1.04, 95% CI: 0.76-1.43) than non-vaccinated chickens. Rearing chicken of different batches in one house was decreased the odds of occurrence of MD by 55% (95% CI: 0.38-0.80) than all-in-all-out, higher in the well-ventilated type farms decreased the odds of occurrence of MD by 60% (95% CI: 0.39-0.80) than in poor ones. Litter management when farmers used chickens as fertilizer has decreased the odds of occurrence of MD by 55% (95% CI: 0.01-0.47) and chickens were tossed into open sheds 40% (95% CI: 0.01-0.31), compared to buried chickens management. The study results indicated that the number of farms where farmers wearing no clothes and shoes (95% CI: 0.10-0.58) were significantly decreased the occurrence of MD by 24% than those where farmers were equipped with clothes and shoes. The study area was highest in West Gojjam (OR: 0.40, 95% CI: 0.27-0.58) and South Gondar (OR: 0.19, 95% CI: 0.13-0.28) compared to North Gondar zone. In conclusion, the present study revealed a high flock and chicken seroprevalence level of MDV among chicken flocks in northwest Ethiopia, suggesting that environmental dust/dander and farm management systems might be a source of this disease for chicken infection. Besides, the observed association of MD, sero-positivity with environmental dust/dander, and farm management systems may suggest the economic importance of the disease for chicken production. Therefore, it warrants control attention to reduce its economic and disease spread burden in the study areas. Further works on the economic impacts, virus isolation, and molecular characterization of the disease are suggested.

Keywords: Chicken, Marek's Disease, Northwest Ethiopia, Risk factors, Sero-epidemiology

INTRODUCTION

Marek's Disease Virus (MDV), recently classified as the Office International des Epizooties list B disease, and is a lymphoproliferative disease of chickens (Kamaldeep et al.,

2007; Couteaudier and Denesvre, 2014). Marek's Disease Virus is a highly cell-associated oncogenic α -herpesvirus that causes immunosuppression (Puro et al., 2018), neurological disorders, chronic polyneuritis, lymphomas in

visceral organs and other tissues, transient paralysis (Atkins *et al.*, 2013; McPherson and Delany, 2016; Wang *et al.*, 2018). The virus is transmitted to inexperienced chickens via the respiratory region (Witter and Burmester, 1967). Macrophages or dendritic cells subsequently transfer the virus to the major lymphoid organs (Woźniakowski and Samorek-Salamonowicz, 2014), where it infects B and T cells (Boodhoo *et al.*, 2019). The route of infection is by inhalation of airborne cell-free virus particles within the contaminated dust and dander, and then shed from the infected host produced in terminally differentiated feather follicle epithelium, into a naive respiratory track (Boodhoo *et al.*, 2016). The virus replicates within the feather follicle and spreads horizontally in poultry houses with dirt and dander (Davidson, 2019). It has a complex life with four main phases: an early cytolytic phase at 2-7 days post-infection (dpi) (Gurung *et al.*, 2017), a latent section around 7-10 dpi, a late lysis section with the presence of tumors that are triggered between the 14 and 21 dpi, and a final proliferation section at 28 dpi. Throughout the primary lysis part, MDV preliminary uses B cells as targets for its replication before targeting activated CD4 T cells to adjust a persistent latent phase (Bai *et al.*, 2019).

In infected chickens, unhealthful field strains of MDV will cause mortality of up to 100% in susceptible flocks; In infected chickens, unhealthful field strains of MDV will cause mortality of up to 100% in susceptible flocks; however, commercial losses due to MD are effectively controlled by the use of antigenically-related live attenuated vaccines (Cadmus *et al.*, 2019; Neerukonda *et al.*, 2019). In Ethiopia, the village system contributes to more than 90% of the national chicken meat and egg output (Ibrahim *et al.*, 2019).

A number of challenges and obstacles warning the success and profitability of both backyard and semi-intensive production have been identified, including infectious diseases, low effort of veterinary services, poor housing, poor biosecurity, predators, as well as the quality and cost of feed (Sambo *et al.*, 2015). The economic encumbrance of MDV hail from both direct losses due to chicken mortality and morbidity (egg production loss) and indirect losses caused by the wide use of vaccines and control measures in the industry (Rozins *et al.*, 2019). Furthermore, immunosuppression resulting in increased coexisting disease and decreases in weight gain are both important indirect effects associated with MDV infection (Atkins *et al.*, 2013). The concentration of MDV in dust can vary by numerous orders of magnitude between farms and within the farms over time. The underlying cause of

this variation is unknown (Kennedy *et al.*, 2018). Periodic disease outbreaks always affect the chicken production system in Ethiopia in different ways by losing almost the entire flock in the village chicken. The other by the constant presence of infectious disease can lead not only to illness and death but also reduction of the product (Habte *et al.*, 2017). The mortality rate due to natural MD challenge varied among four indigenous chickens in Ethiopia by ecotypes Fayoumi (94.8%), Tepi (62.2%), Horro (51.4%), and Jarso (36.3%) (Duguma *et al.*, 2006), and the mortality rate was 46% (Lobago and Woldemeskel, 2004) in non-vaccinated poultry, and about 5% in MDV vaccinated chickens (Duguma *et al.*, 2005). Nevertheless, estimates of overall mortality (56.5%) and morbidity (58.1%) were reported (Asfaw *et al.*, 2021).

Amhara region also has the second (10,368,274) reserves of poultry in Ethiopia (9,983,180 native breeds; 339,046 crossbreeds and 46,049 exotic breeds (Alemu *et al.*, 2008). The majority (94-99%) of the chicken population in Ethiopia was estimated as 49 million in 2011 (Sambo *et al.*, 2015). More than 60% of the Ethiopian families kept, managed, and controlled chickens as a source of income by selling them in cash (Dessie and Ogle, 2001). The reported mortality rate of Lohmann white breeds and Rhode Isle land Red were 29.34% and 16.18%, respectively. The other mortality rate reported was also in exotic chickens in low altitude (52.98%), (48.88%), and mid-altitude (43.25%) (Mazengia *et al.*, 2012). In spite of their low productivity, and poultry health management, especially prevention, control of MDs was poor and particularly no seroprevalence study has been done in the northwest part of the country to address this issue. The findings of previous studies have shown that the seroprevalence of MDV antibodies in chickens ranges from 58% to 74.9%. Within the country, the most important causes of MDV (feeding status, farm type, farm size, production type, rearing method, house sanitation, litter management, carcass management, and farm shoes and clothes within the flocks of chicken are not well documented, and also the association with the seroprevalence of MDV has not been well noted.

When the counts of chickens were high, the farmers did not benefit from the sector disease outbreak in Ethiopia (Yitbarek and Atalel, 2013). However, in the current study area, there was no clear evidence about MD challenges, epidemiological information, sero-prevalence, and status in Northwest Ethiopia.

In Ethiopia, greater efforts have been made to transform the production system into a more commercialized and intensive large-scale system but they

have faced some challenges and serious problems that cause morbidities and mortalities in chickens, especially in the Northwest, Ethiopia. There was no enough data regarding the production constraints, opportunities, and MDV outbreak control for poultry production in the current study.

The current study was the first preliminary one in northwestern Ethiopia. Data on the seroprevalence of MDV in chickens would be caring for planning and implementing precautionary measures for economic impacts. Therefore, the objectives of the study were firstly to estimate the seroprevalence and the possible risk factors of MDV infection in local and exotic chickens in Northwest Ethiopia, and secondly to assess the relationship between sero-positivity of MDV through the environmental and management systems of farms for local and exotic chickens.

MATERIAL AND METHODS

Ethical approval

For the research team members to conduct the current study, after permitted ethical approval and statement given by the University of Gondar, Ethiopia. The current study was reviewed by the Institutional Ethical Review Board of the University of Gondar for its ethical soundness, and it is found to be ethically acceptable. Thus, the Research and Community Service Vice President Office has awarded on R. No. - O/V/PRCS/05/495/2018.

Study areas

Amhara Regional National State is located in the northwestern part of Ethiopia (Fentie et al., 2013). Study zones included North Gondar, West Gojjam, and South Gondar. According to Dachew and Biftu (2014), North Gondar zone is located between geographically coordinates 12.3° to 13.38° north latitudes and 35.5° to 38.3° east longitudes. The altitude ranges from 550 meters below the sea level (masl) which is lowland (in western parts) and 4620 meters above the sea level (masl) which is highland (in the north part). The average annual rainfall varies from 880mm to 1772 mm, which is characterized by a monomodal type of distribution. The mean annual minimum and maximum temperatures are 10°C and 44.5°C in the highland and lowland, respectively. The indigenous chicken flock in the study zone was estimated at 3.75 million. Most of the poultry flocks were found in the highland and mid-highland areas of the region. This was due to the ecosystem and demography variations from low land. In this area, the local chickens were major

poultry flocks in each household level as an income generation with a free-range production system (Fentie et al., 2013). According to Solomon et al. (2013), West Gojjam zone is situated at 11° 09' 60.00" N latitude and 37° 14' 60.00" E longitude with an altitude ranging from 1500 to 3420 m above the sea level. West Gojjam zone is one of the zones in Amhara Region where elevation varies from 1500 to 3500 m.a.s.l. Agriculture is the chief foundation to support the community (Motbaynor et al., 2020). South Gondar zone was encompassed in the study and is located in the Amhara Region, 660 km northeast of Addis Ababa, the Capital of Ethiopia. This Zone is well-known with diverse topography ranging from flat and low grazing land to high cold mountains. The altitude is 1500 to 3,600 m.a.s.l. The average yearly rainfall varies from 700 mm to 1300 mm whereas the average daily temperature is 17°C in 2017 (Alelign et al., 2019).

Study animals

Seroprevalence investigation was conducted in a total of 768 chickens, where 384 local chickens and 384 exotic Sasso T lines (Ruby T) a dual-purpose chicken for meat and eggs, in a farm from Northwestern Ethiopia. Chickens were kept under intensive and extensive poultry farm management in the mixed production system of the area. Chickens, which two and above months of age were included in the sampling process. Farm rearing methods were all-in-all-out and different batches in one house.

Study design and sample size

A present cross-sectional study with a cluster random sampling was undertaken from January 2018 to May 2020. The sample size was calculated using a formula given by Greiner and Gardner (2000). Expected prevalence (Pexp) of 50%, desired absolute precision (d), and a confidence level of 95% was determined by significance. Therefore, 384 Chickens were considered for sampling purposes. However, to account for the design effect associated with the clustering of study units within flocks and locations, the sample size was multiplied by two and the resulting in a total sample size of 768 Chickens.

Blood sample collection and serum separation

Whole blood samples (about 2-3 ml) were collected aseptically through wing vein puncture using 5 ml plain vacuoliner tubes (Birhan et al., 2019). All necessary information related to each chicken included age, breed, sex, vaccine history, feeding status, farm type, farm size, production type, rearing method, house sanitation, litter management, house sanitation, ventilation type, zone, and

farm shoes and cloth were properly labeled on the blood collection tubes and the data recording sheet. The blood samples were then allowed to clot in a slant position for a few minutes and centrifuged at 4000 rpm for 5 minutes to separate the sera. Subsequently, sera were decanted into 1.5 ml Eppendorf tubes and kept at -20°C until the serological analysis for the presence of Marek's antibodies. In the attempts to screen and detect the MDV antibody at the National Veterinary Institute (NVI), all the materials were International Organization for Standardization. Regarding MD, the overall prevalence (Antibody prevalence) using indirect enzyme-linked immunosorbent assay was 59.11%.

Questionnaire survey

A pretested semi-structured questionnaire survey was conducted during the blood sample collection by interviewing owners or attendants to assess the potential risk factors of MDV in chicken flocks. Farm supervisor veterinarians and farm owners were purposively selected. A total of 46 respondents were interviewed using semi-structured questions for epidemiological data regarding total chickens, number of affected birds, number of birds reported dead due to MDV, age, breed, sex, vaccine history, feeding status and farm type, farm size, production type, rearing method, house sanitation, litter management, carcass management, and farm shoes and clothes as a source of the pathogen.

The hypothesized potential risk factors for MDV to assess included having information on the role of vaccine history, feeding status and farm type, farm size, rearing method, house sanitation, litter management, carcass management, and farm shoes and wearing as a source of a pathogen (yes/ no), flock size (50-200, 201–500 and >501), presence of a house as well for sick chickens in or around the farm (yes/no), presence of clothes and shoes for farm management (yes/no), chicken feed sources (properly feed and non-properly feed/millhouse grinder leftover), dead chickens' disposal method (buried, throw away in the field or use as fertilizer), and chicken feed contamination by dust/dander (yes/no). Moreover, the owners/attendants were interviewed about previous experiences of the occurrence of MDV transmissions at the flock/farm or individual chickens' level.

Serological analysis

Marek's disease virus Antibodies were assayed in the collected serum samples using the indirect ELISA kit test (Shenzhen Zhenrui Biotechnology Co., Ltd., China) following the previous reports (Zelnik *et al.*, 2004) and

manufacturer's recommendations. Indirect ELISA tests were detected using the naked eye and stereomicroscope. The presence of an indirect ELISA test was considered as positive at a titer of > 0.2, and showed an antibody concentration equal to or greater than 100 ul. The flocks also were considered when at least one chicken was found positive. The laboratory procedures were performed at the NVI, Serology Laboratory.

Data management and analysis

The data generated was stored in the Microsoft Excel spreadsheet (2010), and analyzed using Stata software Version 16 for windows. The data were summarized using descriptive statistics. Seroprevalence of MDV infection was calculated by dividing the total number of seropositive chickens by the total number of examined poultry. Similarly, chicken flocks' seroprevalence was calculated as the total number of positive divided by the total number of the examined chicken flocks. Mixed effect logistic regression analysis was used to identify potential risk factors associated with MDV. First, invariable logistic regression analysis with the flock as a random effect was performed and potential risk factors (explanatory variables) with p values ≤ 0.25 were screened for the multivariable mixed-effect logistic regression. Before running the multivariable mixed-effect logistic model, the explanatory variables were checked for multi-collinearity using the variance inflation factor (VIF) value of greater than 10 was considered the cut-off point for the collinearity diagnostics (Steinfeld *et al.*, 2015). The associations were considered statistically significant when $P < 0.05$ at 95% confidence level. Odds ratios at a 95% confidence interval were used to express the strength of the risk of the diseases associated with the tested factors. Association of environmental risk factors (feeding status, farm type, farm size, rearing method, house sanitation, litter management, carcass management, and farm shoes and wearing as a source of a pathogen) with sero-positivity for MDV at flock level was analyzed similarly using mixed-effect logistic regression considering reproductive flocks as outcome variables and sero-positivity as the explanatory variable. The cutoff value of the receiver operating characteristic curve in the current study was 0.5% (Figure 1). This indicated that the expected probability from the model may have been a 50% probability of success.

The Area Under the Roc Curve (AUC) is an effective way to summarize the overall diagnostic test. It takes values from 0 (perfectly inaccurate test) to 1 (perfectly accurate test). The AUC can be computed using the general rules,

in these rules the value of AUC (0.5) suggests no discrimination, 0.7-0.8 is considered acceptable, and 0.8-0.9 is considered excellent, and more than 0.9 is considered outstanding (Mandrekar, 2010). In dichotomous outcome (positive/negative test results) was used to plot sensitivity versus 1-Specificity is called receiver operating characteristic (ROC) curve, and AUC, as an effective measure of the accuracy of meaningful interpretations (Hajian-Tilaki, 2013). The AUC was an effective way to summarize the overall diagnostic test. It takes values from 0 (perfectly inaccurate test) to 1(perfectly accurate test). Considering the obtained results of the current study, the AUC of the curve was 0.75. This suggested a 75% chance that the indirect ELISA test was correctly distinguished non-Marek's Disease from Marek's Disease chicken based on the standard of the optical density value with indirect ELISA reader. In this outcome, the result of sensitivity and specificity were 80.18% and (54.46%), respectively, and also the correctly classified was 69.66%. However, in this consequence of score value, the postulation was that indirect ELISA test was subjectively gives considered laboratory results of MD in chickens.

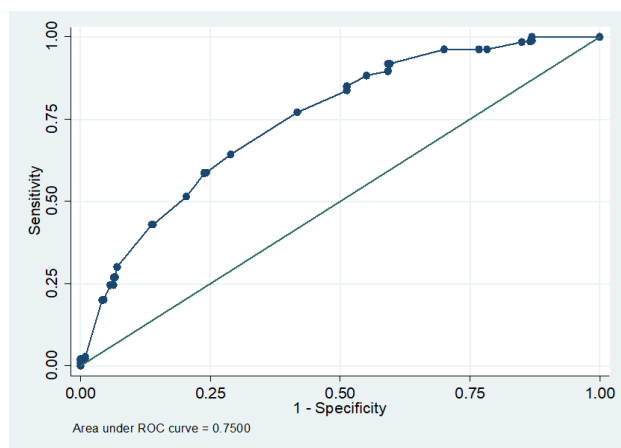


Figure 1. Receiver operator characteristic curve analysis and estimation of the study.

RESULTS

Seroprevalence and host-related risk factor analysis

A total of 768 serum samples were examined and 454 (59.11%) were positive for MDV. As can be seen in Table

1, the results of different age groups indicated a relatively higher prevalence of MDV in adult chickens 61.1% (0.61-0.57), compared to that of young age groups 52.7% (0.45-0.60). Similarly, a relatively higher prevalence of MDV was observed on the extensive farming system (60.4%), as compared to that of the intensive (55.6%) based on chicken farming systems. In the present study, different chicken farm sizes were examined, relatively a higher prevalence of MDV was observed in the medium farm's size (66.2%), as compared to small (60.7%) and large (25%) farms size. Moreover, a relatively higher prevalence of the MDV was observed in the exotic breeds (67.7%) than the local breeds (52.1%). On the other hand, vaccinated chickens higher prevalence (66.3%) than non-vaccinated ones (53.7%).

The results presented in Table 1 indicated that there were statistically significant differences observed ($p < 0.05$) among the prevalence of MDV, according to the age, breed, and vaccine history, feeding status, and rearing methods.

Questionnaire survey related to farm owners/attendants as risk factors for Marek's Disease Virus occurrences

A total of 46 chickens' farm owners/attendants participated in the questionnaire survey aimed at collecting data about potential risk factors for MDV. Out of 46 farm owners'/attendants' respondents, 69.57% did not know concerning the role of house litters and dust/dander as a pathogen source. All of them practiced an extensive management system. Among the respondents, 76.1% had no isolation house in their farms for diseased/sick chickens. The most frequently stated feeding status of chickens was properly fed (83.6 %) followed by non-properly fed (57.2 %). Respondents also stated that litter management, including buried chickens, was 67% whereas chickens that accumulate near the farmhouse were 23.9% and the other that used chickens as fertilizer were 9.1%. The respondents informed about rearing methods, different batches in one house were 71.74% and all-in-all-out methods were 28.3%. The most accurate timetable for farm disinfections was 54.35%, compared to the inaccurate timetable was 45.65%. In the study areas, 10.87% of the farm owners/attendants dress in special clothes and shoes, but 89.13% were not used special clothes and shoes.

Table 1. Invariable and multivariable mixed-effect logistic regression analysis of hosting potential risk factors for Marek's Disease Virus

Variables	Category	Number of examined	Number of positive	Prevalence (%) (95% CI)	Invariable		Multivariable	
					COR (95% CI)	p value	AOR (95%CI)	p value
Breed	Exotic	344	233	67.7 (0.63-0.73)	1.0			
	Local	424	221	52.1 (0.47-0.57)	0.52 (0.39-0.70)	0.000*		
Sex	Male	219	128	58.4 (0.52-0.65)	1.0			
	Female	549	326	59.4 (0.55-0.64)	1.04 (0.76-1.43)	0.812		
Farm Type	Intensive	198	110	55.6 (0.49-0.63)	1.0			
	Extensive	570	344	60.4 (0.56-0.64)	1.22 (0.87-1.86)	0.237		
Farm Size	Small (50-200)	567	344	60.7 (0.57-0.65)	1.0			
	Medium (201-500)	145	96	66.2 (0.58-0.74)	1.27 (0.87-1.86)	0.221		
	Large (>501)	56	14	25 (0.13-0.37)	0.22 (0.12-0.40)	0.000*		
Production Type	Layer	445	278	62.5 (0.58-0.67)	1.0			
	Broiler	170	102	60 (0.40-0.56)	0.90 (0.63-1.29)	0.573		
	Dual	153	74	74 (0.40-0.56)	0.56 (0.39-0.82)	0.002		
Age	Young	184	97	52.7 (0.45-0.60)	1.0			
	Adult	584	357	61.1 (0.61-0.57)	1.41 (1.01-1.97)	0.043	0.41 (0.20-0.87)	0.020
Vaccination history	Vaccinated	436	234	53.7 (40.49-0.58)	1.0			
	Non-Vaccinated	332	220	66.3 (0.61-0.71)	1.70 (1.26-2.28)	0.000*	2.15 (1.54-2.99)	0.000*
Feeding Status	Properly Feed	55	46	83.6 (0.74-0.94)	1.0			
	Non-Properly Feed	713	408	57.2 (0.54-0.61)	0.26 (0.13-0.54)	0.000*		
Rearing Method	All-in-All-out	157	110	70.1 (0.63-0.77)	1.0			
	Different Batches in One House	611	344	56.3 (0.52-0.60)	0.55 (0.38-0.80)	0.002		

COR: Crude odds ratio, AOR: Adjusted odds ratio, CI: Confidence Interval, p values ≤ 0.05 was statistically significant, p values = 0.00* were strongly significant.

Table 2. Invariable and multivariable mixed-effect logistic regression analysis of environmental risk factors for Marek’s Disease Virus

Variables	Category	Number of examined	Number of positive	Prevalence (%) (95 % CI)	Invariable		Multivariable	
					COR (95% CI)	p value	AOR (95% CI)	p value
Ventilation Type	Good	609	378	48 (0.40-0.56)	1.0			
	Poor	159	76	62.1 (0.58-0.66)	0.56 (0.39-0.80)	0.001		
House Sanitation	Good	448	252	47.8 (0.40-0.56)	1.0			
	Poor	320	202	56.3 (0.52-0.61)	1.33 (0.99-1.79)	0.056	0.17 (0.07-0.33)	0.000*
Litter Management	Buried	25	24	63.1 (0.58-0.68)	1.0			
	Use as fertilizer	570	344	96 (0.88-1.04)	0.06 (0.01-0.47)	0.007		
	Accumulate near space	173	86	60.4 (0.56-0.64)	0.04 (0.01-0.31)	0.002		
Zone	North Gondar	256	200	78.1 (0.73-0.83)	1.0			
	South Gondar	256	104	40.6 (0.35-0.47)	0.19 (0.13-0.28)	0.000*	0.05 (0.03-0.10)	0.000*
	West Gojjam	256	150	58.6 (0.53-0.65)	0.40 (0.27-0.58)	0.000*	0.07 (0.03-0.15)	0.000*
Farm Shoes and clothes	Have	40	34	60.4 (0.57-0.65)	1.0			
	Have-No	728	420	85 (0.73-0.97)	0.24 (0.10-0.58)	0.002	0.10 (0.034- 0.27)	0.000*

COR: Crude odds ratio, AOR: Adjusted odds ratio, CI: Confidence Interval, p values ≤ 0.05 was statistically significant, p values = 0.00* were strongly significant.

DISCUSSION

Chicken production is the distinctive local economic development of urban centers through the development of related micro-enterprises wholly or partly responsible for the provision of inputs and processing, packaging, and marketing of outputs as well as the delivery of services to the sector (Ebsa et al., 2019). The current study has indicated the widespread occurrence of MDV infection in the chicken flock in northwest Ethiopia with a high seroprevalence of 59.11%. This coincides with the previous studies in Ethiopia, which similarly reported high seroprevalence of MDV in chickens (Duguma et al., 2005). Similarly, the present findings are compatible with the reports of Oni and Owoade (2009) and Wajid et al. (2013) from Nigeria, and Wajid et al. (2013) from Southern Iraq. However, the current result was higher than other previous reports from the Central African Republic (Snoeck et al., 2012), and lower than reports from Tanzania (Sailen et al., 2017).

In fact, many factors attributed to why the results of the current study differ from other findings. Considering the investigated factors, different diagnostic test kits, the diverse geographical location and climate changes of the study, and the distinctive farm management system can be listed. The variation observed among the different studies in the prevalence of MDV might be due to the difference in the breed, management system, and the epidemiological status of the animals among the different study areas.

Among the risk factors considered in the current study, age, breed, vaccination history, feeding status, rearing method, litter management, ventilation type, zone, and farm shoes and wearing were found to be statistically significant ($P < 0.05$). There was a significant difference in the prevalence among chickens of different ages ($P < 0.05$). The highest prevalence (61.1%) was found in adult chickens, followed by the lowest prevalence (52.7%) in young chickens. The high prevalence of the MDV revealed in adult chickens might be due to the physiology of the exhausted canal which is more dilated, and remains partially open because there have been years of repeated exposure, and it is a chronic disease. Susceptibility to MDV was considered to be much higher in older chickens than in younger ones. Although the age-resistance remained unclear, the immune system maturation likely participated in this event. Marek's disease virus can occur at any time, beginning at 3-4 weeks of age (Ikezawa et al., 2010).

However, there were significant differences in the occurrence of MD between exotic and local breed

outcomes (Table 1). A higher prevalence (67.7%) was recorded in the exotic breed, and a lower prevalence (52.1%) was verified in chickens with the local breed. Due to low genetic-diseases-resistant and other environmental stress, exotic breeds were high exposed than local breeds (Duguma et al., 2005).

Furthermore, non-vaccinated chickens (66.3%) were more susceptible and had the chance of acquiring the MD, whereas the vaccinated chickens (53.7%) less susceptible. However, vaccines are commonly used in the commercial poultry industry because small numbers of doses cannot be purchased for use. The best protection mechanism for the backyard chicken's management systems is to disinfect the area. Predominantly the commercial flocks, it is important to have proper biosecurity to ensure that vaccinated chickens will develop immunity before they are exposed to a severe challenge of MDV (Mazengia, 2012).

Among the environmental risk factors, feeding status, rearing method, ventilation type, litter man management system, and farm shoes and wearing were statistically significant ($P < 0.05$, Table 2). Since chickens are required to be reared separately, they are free from the infected fluff and dust of older birds. Standard hygiene measures are also important, including a thorough clean-out and disinfection of the sheds and equipment among batches of chickens with a disinfectant effective against viruses. Decent nutrition and maintenance of freedom from other diseases and parasites are also vital. These practices will help to maintain the flock's health, and ensure that the birds have optimum resistance against MD infection (Rwuaan et al., 2012). The virus matures into a fully infective, enveloped form in the epithelium of the feather follicle, from which it is released into the environment (Morrow and Fehler, 2004).

The current study showed significant results among various zones ($P < 0.05$). A higher prevalence was recorded in the north Gondar zone (78.1%) followed by the West Gojjam zone (58.6%) and South Gondar zone (40.6%), respectively. According to these studies, this might be associated with the herding practices of chickens. The absence of research-based investigation approaches resulted in a lack of knowledge of the prevalent strain of viruses and information on the overall epidemiological patterns of the MDV (Witter et al., 1970).

CONCLUSION

In conclusion, the current preliminary study revealed a high seroprevalence of Marek's disease (MD) in the chicken flocks in northwest Ethiopia, suggesting that by

applying proper farm management systems with biosecurity, such as Good rearing method, litter management, and follow up seasonal vaccines, the disease can be prevented and controlled. The study also demonstrated that sero-positivity for MDV was associated with age, breed, and vaccine history, feeding status, rearing method, litter management, ventilation type, zone, and farm shoes and wearing with an important economic significance for poultry farm owners. Therefore, it warrants control to reduce its economic and disease burden. Further works on the virus isolation and economic impacts are needed in the study area and it is also given attention to further investigation on the effectiveness of vaccines and their delivery system. In order to know the circulation virus and strain type, the molecular study of the diseases is suggested.

DECLARATIONS

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Authors' contributions

Mastewal Birhan, Nega Berhane, Molalegne Bitew, Esayas Gelaye, and Takele Abayneh are substantial contributors to the conception and design, acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content. Belayneh Getachew, Aragaw Zemene, Kassahun Birie, and Wudu Temesgen are given some technical comments. Mastewal Birhan, Nega Berhane, Molalegne Bitew, Esayas Gelaye, and Takele Abayneh approved the final draft of the manuscript to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Competing interests

All authors declare that they have no competing interests.

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