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Prevalence, Gross Pathology, and Histopathology of Marek's Disease in Backyard Chickens in Northeastern Tunisia

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ABSTRACT

Marek's disease (MD) is a common worldwide lymphomatous and neuropathic disease of chickens. Infection can cause significant losses in chicken production due to high mortality and morbidity. The present study aimed to determine the prevalence of MD in backyard flocks in the Grand-Tunis region of northeastern Tunisia and to analyze clinical cases over an eight-year and three-month period, from September 2012 to December 2020. A total of 798 cases were received for necropsy examination in the avian clinic of the National School of Veterinary Medicine of Sidi Thabet, Tunisia. Among these, chicks suspected of having MD underwent clinical observation, postmortem examination, and histopathological analysis. The results showed that 61 chickens (7.64%) were suspected to have MD. Clinical and postmortem examinations revealed different forms of MD including visceral (31 cases), mixed (20 cases), and nervous forms (10 cases). Postmortem examinations showed two types of lesions including hypertrophy and lymphomatous tumors. The highest frequencies of lesions were noted in the liver (74%), spleen (62%), sciatic nerves (48%), lungs (36%), and kidneys (31%). Hypertrophy predominated in the spleen (49%), sciatic nerves (48%), liver (28%), kidneys (25%), lungs (21%), proventriculus (18%), and gonads (17%). Conversely, lymphomatous tumors were more frequently observed in the liver (46%), heart (23%), lungs (15%), and spleen (13%). Histopathological investigations revealed pleomorphic infiltrations with lymphocytes and plasmocytes in visceral organs, sciatic nerves, and the skin. High histological scores were recorded in the liver, spleen, lungs, kidneys, and heart. The current study confirmed endemic MD in backyard chicken populations in Grand-Tunis région and confirmed that it can be a serious threat to poultry health in the study area.

Keywords: Backyard chickens, Clinical examination, Histopathology, Lymphoma, Marek's disease, Postmortem examination

INTRODUCTION

The traditional poultry sector plays a vital role in rural zones of Tunisia. It provides eggs and meat, as high-quality sources of animal proteins for both local market and household consumption. According to the latest official statistics from GIPAC (2010), this sector contributes an average of 7% to the national poultry production. Backyard poultry typically consists of small flocks of different poultry species reared under traditional conditions. Productivity in these flocks is often unsatisfactory because of serious health problems, malnutrition, and poor management conditions. In addition, potential threats to productivity, such as poor genetic potential due to lack of selection and predation, as well as infectious and non-infectious diseases, should be considered. Chickens raised in free-range systems are exposed to constant risks of infection by several pathogens, such as the Marek's disease virus (MDV).

Marek's disease (MD) is a worldwide viral and highly contagious neoplastic disease in poultry. The causative agent is Gallid-Herpesvirus 2 (GaHV-2), classified as a member of the family *Herpesviridae*, subfamily *alpha-herpervirinae,* and genus *Mardivirus*. Of the three

recognized three serotypes of MDV, only serotype 1 contains viral strains capable of inducing tumors (Morrow and Fehler, 2004).

Marek's disease is diagnosed in poultry-producing countries throughout the world. However, the incidence prior to the availability of vaccines was not uniform. Economic losses caused by MDV infections were especially high in intensive systems. The virus is transmitted through direct and/or indirect contact between chickens, most commonly via the airborne route (Abdul-Careem et al., 2009a). Fully infectious virus particles are replicated in the epithelial cells in the keratinizing layer of the feather (Abdul-Careem et al., 2009b). These cells serve as a source of environmental contamination. MDV associated with feathers and dander is infectious for at least several months at 20°C to 25°C and for years at 4°C (Calnek and Witter, 1997). Chickens can act as asymptomatic carriers and transmit the virus. The resilience of the virus and the ongoing shedding by infected birds make its prevalence readily understandable. To date, vertical transmission of MDV has not been confirmed. Similarly, transmission of the virus from breeder hens to progeny through external eggshell contamination has remained uncertain due to poor virus survival under temperature and humidity conditions of the incubation process (Pohjola et al., 2015; Mete et al., 2016).

The Marek's Disease Virus infections are characterized by T-cell lymphoma of peripheral nerves, viscera, skin, and eyes. Morbidity and mortality rates range from 5% to 30% when hypervirulent strains are incriminated. MD infection can occur from 3-4 weeks of age in mature chickens; however, clinical manifestations are often described at 12-30 weeks of age. The immunosuppressed condition due to MDV is a potential cause of vaccination failure against other contagious diseases, increasing the susceptibility of chickens to infection with other pathogens (Gimeno and Schat, 2018).

Symptoms of MD include depression, stunting, lethargy, characteristic unilateral paralysis of the legs, and mortality (Calnek and Witter, 1997; Nair, 2018). Detection of the virus, viral antigens, or nucleic acids in the absence of clinical disease does not confirm the occurrence of MD, resulting from the ubiquitous character of MDV and, subsequently, the presence of the virus in many poultry farms (Nair, 2018). Clinical signs of MD associated with lymphoma formation in multiple organs as well as enlarged peripheral nerves may suffice to make a tentative diagnosis (Nair, 2018). However, confirmation of the diagnosis can be performed by immunohistochemistry, histopathology, and polymerase chain reaction (PCR) (Calnek and Witter, 1997; Nair, 2018).

In Tunisia, MD has been documented in commercial poultry flocks, with clinical forms confirmed in broiler chickens and layer hens. Furthermore, the disease has been rarely reported in commercial meat-type turkeys and broiler breeders. However, there are few special reports on MD in backyard chickens (Kaboudi et al., 2019).

The control of MD infections is based on biosecurity measures and vaccination. Currently, vaccines provide effective prevention against MD. In Tunisia, different vaccine strains are available, including herpesvirus of turkey (HVT), Rispens, and SB-1. Vaccination is only provided for *Gallus gallus* breeders and layer hen flocks and is not routinely performed in commercial broiler chickens or turkey flocks.

The present study aimed to examine the prevalence of MD in free-range chickens received at the avian clinic of the National School of Veterinary Medicine of Sidi Thabet from various regions of Tunisia. Diagnosis was based on clinical signs, postmortem examination, and histopathological analyses of different tissue samples.

MATERIALS AND METHODS

Ethical approval

The experiment was approved by the Institution of Agricultural Research and Higher Education, National School of Veterinary Medicine of SidiThabet, University of Manouba, 2020 Sidi Thabet, Tunisia. It was conducted between September 2012 and December 2020.

Study area

The present study was conducted on backyard poultry flocks located in the "Grand-Tunis" region, comprising four governorates included Ariana (36°51′45″N, 10°11′44″E), Ben Arous (36°44′50″N, 10°20′0″E), Manouba (36°48′28″N, 10°6′4″E) and Tunis (36°48′23″N, 10°10′54″E; Figure 1). These governorates are divided into 48 districts, covering an area of 2.726 km^2 and a total population of 2.731.507 inhabitants. The agricultural surface and humid zone surface range from 24.3% (Tunis) to 78.3% (Manouba) and from 0.6% (Manouba) to 18.8% (Tunis), respectively. The mean annual rainfall and temperature range from 275 to 515 mm and 15.2 to 24.9°C, respectively. The average annual humidity is approximately 70%.

Animals

The current study was carried out between September 2012 and December 2020. A total of 798 chickens (dead and live), coming from 370 flocks and aged between 1 month and 3 years old, were admitted at the avian clinic of the National School of Veterinary Medicine of Sidi Thabet, Tunisia, for postmortem examination. The average weight of chickens ranged from 800 gr to 2.5 kg. Live chickens showed lethargy, anorexia, poor growing rate, respiratory distress, diarrhea, and leg paralysis. For each case, an individual data form was completed, and epidemiological information was recorded and analyzed to facilitate diagnosis. All chickens were obtained from flocks reared under traditional conditions. None of the flocks had a history of MD vaccination.

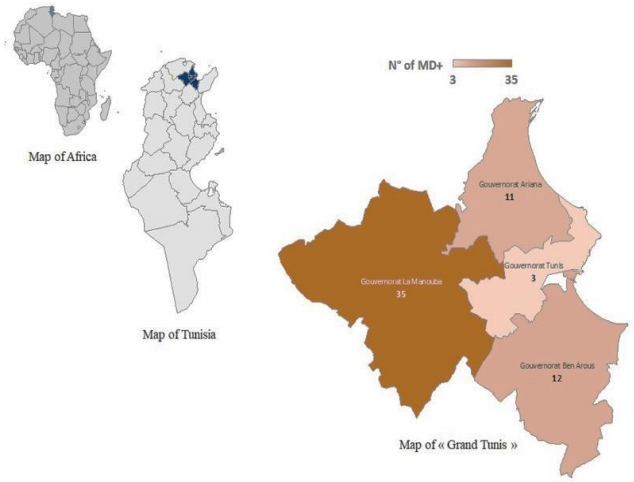

Figure 1. Tunisia and Grand-Tunis region showing the geographic location of sampled birds suspected of Marek's disease

Clinical and necropsy examinations

Live chickens were examined and their clinical signs were registered. Postmortem macroscopic examinations were conducted following standard protocols within 24 hours of death. After external examination, the general cavity was explored to remove and examine the heart, the liver, the digestive tract, lymphoid organs, the trachea, and the lung. The reproductive tract of adult hens was also removed. Finally, the kidneys, the locomotor systems, and the nervous systems were explored. All organs were closely dissected and examined for macroscopic changes relevant to MD (Schwartz and Bickford, 1986).

Tissues and organs suspected of being affected by MD were sampled for histopathological investigations. A total of 196 samples were fixed in 10% formalin solution and sent to the histology laboratory at the National School of Veterinary Medicine of Sidi Thabet, Tunisia, for microscopic examination.

Histopathology

Small tissue samples (0.5 × 0.5 cm) were collected from the liver (45 samples), kidneys (19 samples), proventriculus (12 samples), heart (14 samples), spleen (38 samples), lungs (22 samples), gonads (12 samples), pancreas (3 samples), skin (2 samples), and the lumbosacral plexus and sciatic nerves (29 samples). The samples were fixed in 10% neutral buffered formalin for 48 h. They were dehydrated in graded alcohol series, cleared in toluene, and then processed by the standard paraffin embedding technique. The slices were cut at 4 µm thick and mounted on microscope slides. They were stained with hematoxylin and eosin (HE) and finally examined under an optical microscope (Leitz, Germany) at 10x, 40x, and 100x magnifications for the detection of lesions. Histological scores designed for the present study were adopted from the methodology described by Mete et al. (2016). The severity of lesions was categorized as follows included few, mostly perivascular infiltration and/or scattered lymphocytic infiltrations (+), moderate numbers of lymphoid cells (++), and large multifocal to coalescing sheets of lymphocytes modifying the tissue architecture (+++).

Statistical analysis

Statistical analysis was performed using the SPSS software package (version 16.0, Chicago, SPSS Inc., 2007) for Windows. The Pearson chi-square test was used to evaluate the relationship between epidemiological criteria and the diagnosis of MD in the examined chickens at a threshold value of 5%.

RESULTS

Epidemiological and clinical signs

Out of 798 examined chickens, 61 (7.64%) were positive for MD. The highest MD prevalence was recorded in 2016, with 19.57% (18 cases out of 92), followed by 2015 with 13.16% (10 cases out of 76), and 2017 with 10% (9 cases out of 90). No cases of MD were diagnosed in the samples collected in 2014 (Table 1).

Marek's disease was concurrently diagnosed in all the selected governorates in Tunisia. The highest prevalence was observed in Manouba (35 cases/354; 9.89%),

followed by Ben Arous (12 cases/139; 8.63%). Conversely, the lowest prevalence rates were reported in Ariana (4.52%) and Tunis (4.84%). The disease was reported in young chickens (broilers) aged under 6 months (32 chickens; 5.18%) and adult chickens aged over 6 months (29 chickens; 16.11%; $p < 0.05$). The prevalence of MD infection was 8.84% (38 chickens) and 6.42% (23 chickens) in females and males, respectively ($p > 0.05$; Table 2). Clinical findings revealed that the visceral form of MD was predominant, with 31 cases (50.82%), followed by the mixed form with 20 cases (32.78%), and the nervous form with 10 cases (16.4%).

No specific clinical signs were noted in chickens with the visceral form (32 cases). The most commonly observed symptoms included prostration, respiratory distress, and diarrhea. In contrast, chickens with the nervous form predominantly showed leg paralysis (29 cases; Figure 2).

Necropsy findings

Lesions were observed in several viscera and tissues. The organs and tissues examined in the current study showed two types of lesions including hypertrophy (present in all samples except the heart, pancreas, and skin) and lymphomatous tumors (present in all samples except the nerves; Table 3).

The highest frequencies of lesions were noted in the liver (74%), spleen (62%), sciatic nerves (48%), lungs (36%), and kidneys (31%). Lesions were also observed, though less frequently, in the heart (23%), proventriculus (20%), and gonads (20%). Tumoral lesions in the pancreas (5%) and skin (3%) were rare.

Regarding the types of lesions, hypertrophy predominated in the spleen (49%), sciatic nerves (48%), liver (28%), kidneys (25%), lungs (21%), proventriculus (18%), and gonads (17%). In contrast, lymphomatous tumors were more frequently detected in the liver (46%), heart (23%), lungs (15%), and spleen (13%). No lymphomas were identified on the surface of the nerves.

Whereas organs with hypertrophy were pale and exhibited diffuse tumoral infiltration, viscera with nodular lymphomas displayed deformation and irregular surfaces (Figure 3).

Based on the findings, the mixed form was diagnosed in 20 chicks. The association between visceral and nervous lesions was the most common (17 cases). However, the nervous form associated with the cutaneous form was noted in one chicken. In addition, the visceral form associated with the cutaneous form was noted in another. Finally, the simultaneous evolution of the nervous, visceral, and cutaneous forms was observed in one chicken. No ocular, intestinal, mesenteric, or muscular tumoral lesions were identified in the present study.

Histopathological investigations

The histopathology of affected organs (196 samples) showed a marked cellular polymorphic lymphomatous infiltration. Tumoral lymphocytes and plasmocytes were arranged in multifocal or diffuse patterns. Pleomorphic neoplastic infiltration, characterized by cells of different sizes (small, medium, and large lymphocytes, as well as numerous lymphoblasts), was observed in different viscera and tissue samples (Figures 4 and 5). Histologic scores, based on lymphoproliferative changes, varied from mild (+) and moderate (++) to severe (+++). High histologic scores were most frequently observed in the liver, spleen, lungs, kidneys, and heart. The results regarding lesion severity are detailed in Table 4.

Figure 2. Backyard chickens suspected of Marek's disease with leg paralysis, Tunisia

Table 1. The distribution of Marek's Disease in backyard chickens of Grand-Tunis, North-Est of Tunisia during 2012-2020

Years	Total cases	N° negative MD	N° MD cases	Prevalence (%)
2012	84	83	1	1.19%
2013	95	91	4	4.21%
2014	88	88	0	0.00
2015	76	66	10	13.16%
2016	92	74	18	19.57%
2017	90	81	9	10.00%
2018	104	98	6	5.77%
2019	106	98	8	7.55%
2020	63	58	5	7.94%
Total	798	737	61	7.64%

Table 2. Prevalence of Marek's Disease in backyard chickens according to sample location, age, and sex of diseased chickens (Tunisia, 2012-2020)

		MD -	MD +	The total of examined animals	Prevalence (%)	Chi-square	p-value
Location	Ariana	232	11	243	4.52%	6.752	0.1
	Ben Arous	127	12	139	8.63%		
	Manouba	319	35	354	9.89%		
	Tunis	59	3	62	4.84%		
Age	Young	586	32	618	5.18%	23.602	0.001
	Adult	151	29	180	16.11%		
Sex	Female	402	38	440	8.84%	1.369	0.3
	Male	335	23	358	6.42%		

Table 3. Postmortem lesions types in different viscera and tissues of backyard chickens infected with Marek's Disease (Tunisia, 2012-2020)

Type of lesion	Liver	Spleen	Lung	Proventriculus	Kidney	Gonads	Heart	Skin	Pancreas	Nerve
Hypertrophy	17 (28%)	30 (49%)	13 (21%)	11 (18%)	15 (25%)	10 (17%)	0	0	0	29 (48%)
Lymphomatous tumors	28 (46%)	8 (13%)	9 (15%)	1 (2%)	4 (6%)	2 (3%)	14 (23%)	2 (3%)	3	0
Total (%)	45 (74%)	38 (62%)	22 (36%)	12 (20%)	19 (31%)	12 (20%)	14 (23%)	2 (3%)	3 (5%)	29 (48%)

Table 4. Histological score of lymphocytic infiltration in different organs and tissues (n = 61) of Marek's Disease in backyard chickens (Tunisia, 2012-2020)

Visceral organ/tissue	Mild (+)		Moderate (++)		Severe (+++)		Total	
	N°	%	N°	%	N°	%	N°	%
Liver	8	13%	17	28%	20	33%	45	74%
Spleen	4	7%	21	34%	13	21%	38	62%
Nerve	12	20%	10	16%	7	11%	29	48%
Lung	5	8%	6	10%	11	18%	22	36%
Kidney	4	7%	3	5%	12	20%	19	31%
Heart	4	7%	3	5%	7	11%	14	23%
Proventriculus	3	5%	5	8%	4	7%	12	20%
Gonad	2	3%	6	10%	4	7%	12	20%
Pancreas	0	0	2	3%	1	2%	3	5%
Skin	0	0	1	2%	1	2%	2	3%

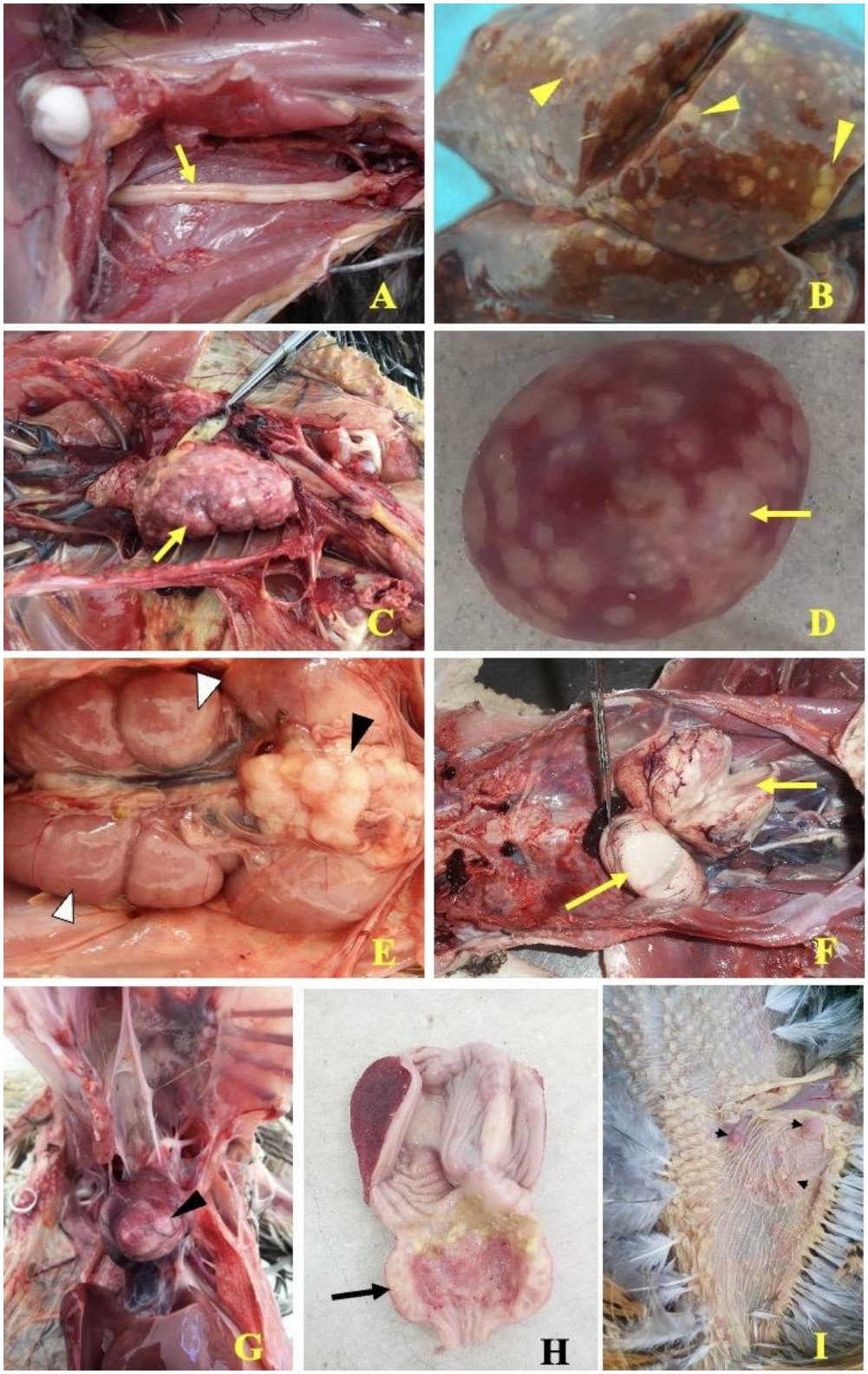

Figure 3. Gross pathology of suspected Marek's disease in backyard chickens in Tunisia during 2012-2020.
A: Sciatic nerve: hypertrophy (yellow arrow). **B:** Liver with numerous lymphomatous tumors (yellow head arrow). **C.** Lung: pale and hypertrophy caused by lymphomatous tumors (yellow arrow). **D:** Spleen: hypertrophy with lymphomatous tumors (yellow arrow). **E:** Kidneys and ovary: pale and hypertrophied (white head arrow). Ovaries appeared with lymphomatous tumors (blackhead arrow). **F:** Testis: dyssimetric and hypertrophied testicles (yellow arrows). **G:** Heart: lymphomatous tumors (blackhead arrow) **H:** Proventriculus: hypertrophied wall (black arrow). **I:** Skin: numerous lymphomatous tumors with varied sizes (blackhead arrow).

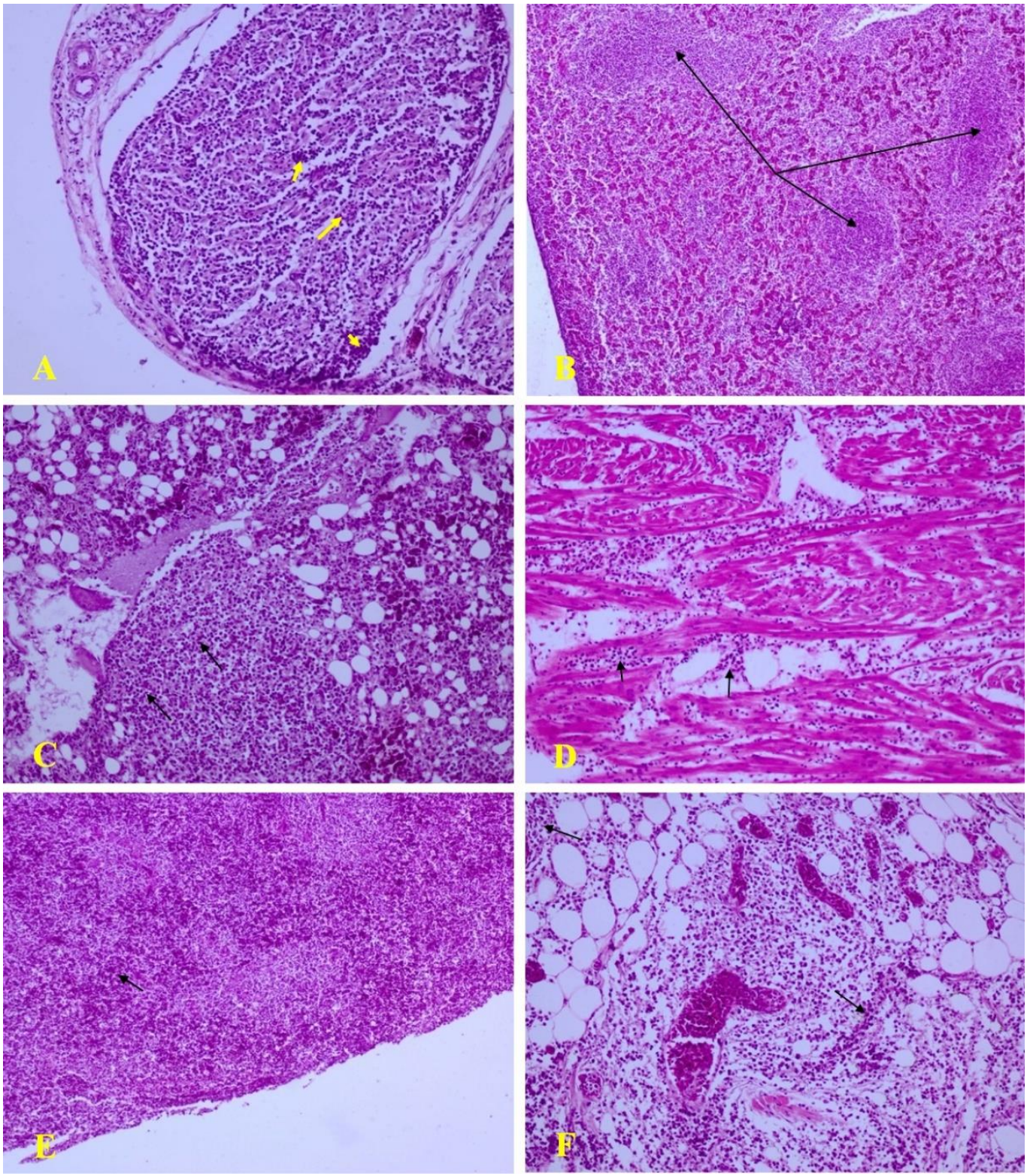

Figure 4. Microscopic lesions were consistent with Marek's disease in backyard chickens in Tunisia from 2012 to 2020.
A: Sciatic nerve: Pleomorphic lymphocytes and plasmocyte infiltrations (yellow arrows; Lesions type A; H&E x 200). **B:** Liver: pleomorphic lymphocytes and plasmocytes infirtations (black arrows; histologic score: +++; H&E x 100). **C:** Lung: pleomorphic lymphocytes and plasmocytes infirtations (black arrows; histologic score: +++; H&E x 200). **D:** Heart: Pleomorphic lymphocytes and plasmocytes infirtations (black arrows; Histologic score: ++; H&E x 200). **E:** Spleen: Pleomorphic lymphocytes and plasmocytes infirtations (black arrow; Histologic score: +++; H&E x 100). **F:** Skin: Pleomorphic lymphocytes and plasmocyte invitations (black arrows; Histologic score: ++; H&E x 200).

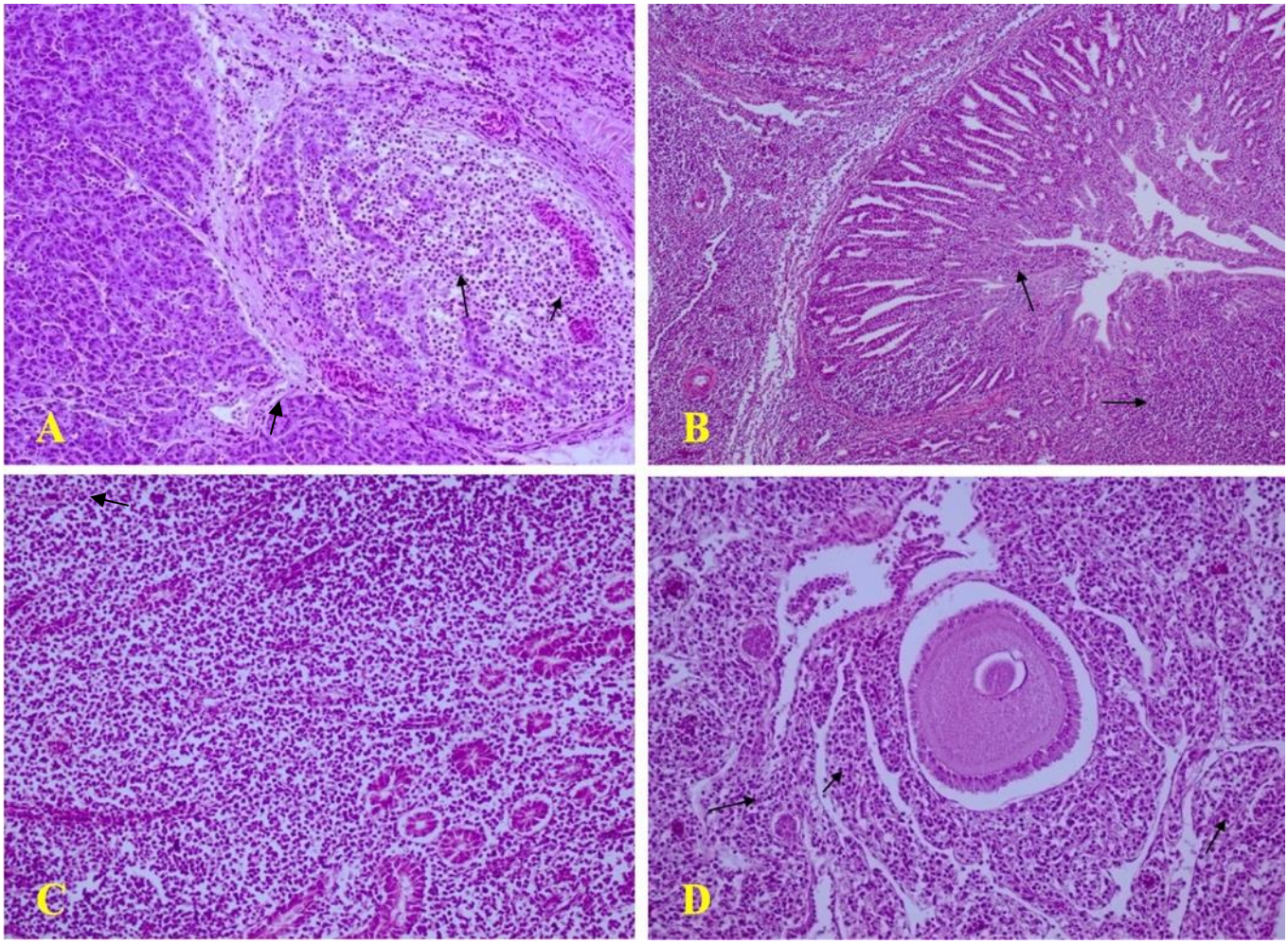

Figure 5. Microscopic lesions are consistent with Marek's disease in backyard chickens in Tunisia from 2012 to 2020. **A:** Pancreas: Pleomorphic lymphocytes and plasmocyte infiltration (black arrows; Histologic score: ++; H&E x 200). **B:** Proventriculus: Pleomorphic lymphocytes and plasmocyte infiltration (black arrows; Histologic score: ++; H&E x 40). **C:** Kidney: Pleomorphic lymphocytes and plasmocyte infiltrations (histologic score: +++; H&E x 200). **D:** Ovary: Pleomorphic lymphocytes and plasmocyte infiltration (black arrows; Histologic score: +++; HE x 200).

DISCUSSION

The positive cases of MD were diagnosed in the present study based on typical gross pathology and histopathological investigations, which remain critical for diagnosing field cases of MD (Pohjola et al., 2015; Wen et al., 2018; Brochu et al., 2019).

Out of the 798 necropsied free-range chickens, 7.64% were positive for MD. In the current study, the prevalence was lower than 13.96% reported recently by Azeem et al. (2023) in backyard chicken flocks in Pakistan. However, Sani et al. (2017) reported an even lower prevalence of 6.52% in Nigerian poultry, with Indigenous chickens showing a notably low rate of 0.43%.

Previous studies have reported MD as the most prevalent viral disease among backyard chickens in several regions. For instance, Crespo and Senties-Cue (2015) found a prevalence of 17.7% in chickens received at the Avian Health and Food Safety Laboratory in California from 2010 to 2014. Pohjola et al. (2015) reported a higher prevalence of 26.51% in Finland from 2000 to 2011, and Brochu et al. (2019) observed an 11% prevalence in submissions to the animal health laboratory at the Ontario Veterinary College, Canada, between 2015 and 2017. The variation in MD prevalence across studies can be attributed to differences in geographic location, sample sizes, biosecurity practices, chicken ecotypes, vaccination status, and the virulence of MDV strains.

Marek's Disease typically affects chickens older than 20 weeks. However, mortality due to MD lymphoma can be observed as early as 7-12 weeks (Calnek and Witter, 1997). In the present study, cases of MD were more

frequently diagnosed in adults above 24 weeks of age, a finding that contrasts with the results of Duguma et al. (2005),. who reported a higher incidence in Ethiopian local chickens aged 14- 20 weeks.

Gross pathology findings from this study showed lymphoma and/or diffuse tumoral infiltration in several visceral organs (liver, heart, spleen, kidney, gonads, and pancreas) of the chicks, as well as the involvement of sciatic nerves and skin. These observations are consistent with the findings reported in previous studies (Lobago and Woldemeskel, 2004; Haridy et al., 2019; Kaboudi et al., 2019; Birhan et al., 2023).

The predominance of the visceral form of MD (50.82%) in this study aligns with observations by Duguma et al. (2005). In general, lesions in visceral organs were observed in chickens with acute clinical signs of MD. Nair (2018) suggested that acute and /or visceral forms of the disease were correlated to the emergence of virulent viral strains of MDV. No tumor lesions were detected in the intestines, mesentery, and primary lymphoid organs, consistent with the findings of Sani et al. (2017), Ho et al. (2021), and Dwinna et al. (2023).

Paralytic symptoms, characteristic of the chronic form of MD, were associated with enlarged, pale, or grayish nerves that lacked cross-striations. These findings mirror those described by Crespo and Senties-Cue (2015). The mixed forms of MD described in this study (20 chickens) were in agreement with those reported by Duguma et al. (2005).

Multifocal nodular cutaneous lymphomas observed in this study resembled those described by Adedeji et al. (2019). Similar gross pathological lesions, particularly cutaneous and visceral forms, can be confused with avian leucosis and reticuloendotheliosis (Nair and Fadly, 2013). While skin lymphomas were common in MD cases, they were rare in avian leucosis, as compared to MD. In addition, visceral lymphomas in avian leukosis were soft, smooth, and glistening, with a grayish to creamy-white appearance (Nair and Fadly, 2013). Also comparably, the skin lesions in reticuloendotheliosis were lymphocytic infiltrates in and around feather follicles and the skin of the head and visceral lymphomas were nodular and firm. Bursal lymphomas, which were pathognomonic lesions in the diagnosis of avian leucosis and reticuloendotheliosis (Nair and Fadly, 2013), were not observed in this study.

The variation in the severity of symptoms, lesions, and mortality and morbidity rates observed in MD cases might be related to genetic resistance of chicks, age, immune status, infection pressure, and the virulence of MDV circulating strains (Nair et al., 2020). This was in agreement with the findings of Duguma et al. (2005) and Adedeji et al. (2019). Differential diagnosis between MD and lymphoid leukosis can be based on the morphology of lymphoid cells. In leucosis, the lymphocyte cells were homogeneous in shape with the constituent cells being lymphoblast, which were characterized by more cytoplasm and visible cell nuclei (Haridy et al., 2019).

The histopathological features described in different visceral organs were characteristic of MD (Calnek and Witter, 1997; Nair et al., 2020). In contrast, the predominant cells usually observed in avian lymphoid leucosis and reticuloendotheliosis are uniform, blast-like, pyroninophilic cells with B-cell markers (Nair and Fadly, 2013). The pleomorphic cells, neoplastic lymphocytes, and plasmocyte infiltrations observed in different organs and tissues in this study strongly suggest field infection with MD. The lesions in the present study were well-attached to those described in several previous reports (Lounas et al., 2021; Viet Thu et al., 2022; Azeem et al., 2023). Vieira-Pinto et al. (2003) noted that pleomorphic tumoral infiltrations revealed in nerves and viscera could serve as confirmatory evidence of MD. Pancreatic lesions, characterized by the proliferation of lymphocyte cells, were similar to those mentioned by Haridy et al. (2019). The massive proliferation of lymphocytes plays a significant role in the immune system's response in animals infected with MDV (Ali et al., 2014).

Overall, high histological lymphocytic infiltration scores were attributed to animals with gross tumors in the examined viscera. These findings are in agreement with the results of Mete et al.'s (2016) study. Severe histological lesions observed in this study might be explained by the circulation of extremely virulent MDV. Indeed, Lachheb et al. (2020) confirmed the circulation of very virulent MDV in Tunisian broiler flocks. In their study, MDV was detected directly from conserved tissue samples (liver, spleen, heart, and kidneys) using PCR, although no histological examination was performed.

CONCLUSION

The gross pathology and histological lesions observed in this study confirm that MDV has been circulating in traditional poultry flocks under study over the last decades (2012-2020). The application of rigorous biosecurity measures and regular vaccination against MD should be improved, particularly in nearby commercial poultry flocks located in the studied region, to reduce the risk of

contamination. Further molecular studies were also needed to characterize circulating strains in free-range chickens. In addition, a comparative molecular study on MDV isolated from industrial poultry flocks can be very useful to understanding the potential epidemiological role of backyard chickens in the spread and persistence of MD infection.

DECLARATIONS

Funding

No available funding was received for the study.

Competing interests

There are no conflicts of interest declared by the authors.

Ethical considerations

The article was written originally by authors from obtained data in current study. The content of the manuscript was checked for plagiarism before submission to the journal.

Authors contributions

KK performed the experimental protocol, interpreted statistical and post-mortem results, prepared figures and tables, and took the lead in drafting the manuscript. EM performed a necropsy examination, collected epidemiological information and samples, and drafted several sections of the manuscript. AA performed the histopathological analysis and prepared figures of histological sections. All authors read and approved the final manuscript.

Availability of data and materials

The data generated and analyzed during this study are available from the corresponding author upon reasonable request.

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