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Original Article

Pathological and Immunohistochemical Examinations in Chukar Partridge (Alectoris chukar) of Wild and Captive Populations

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Partridge, diagnosis, contagious disease, game bird, pathology.

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ABSTRACT

The aim of this study was to evaluate the health situation of chukar partridges through several diagnostic methods. We investigated a total of 224 birds in eighteen populations throughout Turkey, fourteen wild and four captive. The molecular sexing method was used for gender identification. Clinically, traumatic lesions and inflammatory reactions were the most commonly observed in the eyes and extremities of the Chukar Partridges in the breeding stations. At necropsy, the most common findings were enteritis and liver lesions. At the histopathological examination, pneumonia, anthracosis, and inflammatory changes in the digestive system were among the common findings. Interestingly, liver parasites were found in wild samples. At the immunohistochemical examinations, the tissues were evaluated for Marek's disease (MD), Adenovirus, Avian mycobacteriosis (AMB), and Aspergillosis. While MD and AMB were not found in breeding stations, we determined them from wild populations. Aspergillus was found in both wild and captive populations; Adenovirus antigens were found only in breeding stations. When the captive and wild populations were evaluated together, MD 1.3%, AMB 0.9%, Aspergillosis 1.3%, Adenovirus 1.8% were found. The relation between sex and diseases was also examined. As a result, the data of this study showed that chukar partridges from both breeding stations and wild populations are not an important source of diseases, but especially released partridges from breeding stations may carry some microorganisms. For that reason, captive populations should be regularly monitored for contagious diseases.

INTRODUCTION

The chukar partridge (*Alectoris chukar*) is a popular medium-sized game bird belonging to the Phasianidae family. The species' natural distribution is the Middle East and Asia, including Palestine, Lebanon, Turkey, Iran, Afghanistan, Pakistan, and India, along with the inner ranges of the Western Himalayas through Nepal. Chukar partridges are grey-brown birds above with buff bellies. This partridge has well marked black and white bars on the flanks, and a black band runs from the forehead across the eye and runs down the head to form a necklace surrounding a white throat. Male and female plumage patterns are similar, but males are slightly larger than females and have spurs. Molecular sexing methods are used for identification of the gender because spurs are observed in elder females too (Rasmussen & Anderton, 2005; BirdLife International, 2016).

Adenoviruses are non-enveloped double-stranded DNA viruses belonging to the Adenoviridae family (Benko & Harrach, 2013). Avian adenoviruses are common viruses that cause infections in a variety of avian species throughout the world. The Adenoviridae family is classified

into three genera, Aviadenovirus, Atadenovirus and Siadenovirus (Benko & Harrach, 2013). Most of the characterized adenoviruses isolated from domestic and wild birds are classified into the Aviadenovirus genus. All avian species of all ages may be susceptible to the virus (McFerran *et al.*, 1976). However, the possibility of occurrence of clinical diseases is not well known. They are commonly isolated in apparently healthy birds. However, they are the causative agent of important diseases such as inclusion body hepatitis, bronchitis, and hydropericardium syndrome in different bird species (McFerran *et al.*, 1976).

Marek's disease (MD) is a common, highly contagious, and important poultry disease caused by the Marek's disease virus (MDV), belonging to the Herpesviridae family. MDV serotype 1, also known as gallid herpesvirus 2, can result in important economic losses in the poultry industry throughout the world (Kennedy *et al.*, 2017). Different MDV pathotypes have been identified based on morbidity and mortality rates (Gimeno *et al.*, 1999). The disease is characterized by T-cell lymphoma, immunosuppression, neurological disorders and the enlargement of peripheral nerves, only being controlled by mass vaccination. Diagnosis of the disease may be made by history, clinical symptoms, necropsy, and histopathological findings (Morrow & Fehler, 2004). The global presence of MDV infection may be the product of natural reservoirs located in backyards and migratory birds, including roul roul partridges (Murata *et al.*, 2012; Haesendonck *et al.*, 2015).

Avian mycobacteriosis (AMB) is one of the most important insidious, chronic, and contagious diseases that affect all domestic, wild, and pet bird species worldwide (Fulton & Thoen, 2003; Millan, 2009). Several mycobacteria can be isolated as the etiological agent of the disease. AMB is most often caused by *Mycobacterium avium* belonging to serotypes 1, 2, 3, and 6, and less frequently by *Mycobacterium genavense* and *Mycobacterium bovis* (Fulton & Thoen, 2003; Converse, 2007). *Mycobacterium avium* is a Gram positive, aerobic, and saprophytic bacillum with a slow glycerol-dependent growth (Converse, 2007). The agent can cause disease of different severities in birds, depending on susceptibility (Millan, 2009).

Due to the fact that aspergillosis is the primary mycosis that affects birds and animals, including humans, it has a very specific place in both veterinary and human medicine (Seyedmousavi *et al.*, 2015). There is a wide range of species that are susceptible to this infection, including domestic and wild animals living in captivity or in the wild, because of the ubiquity of Aspergillus in different indoor and outdoor environments. The capacity of this opportunistic mold to grow effectively in birds after the inhalation its spores can result in a high mortality rate. Despite the severity of illnesses, which can have a high death rate, and the relatively significant number of case reports and the amount of experimental data, particularly that derived from poultry species, the pathophysiology of avian aspergillosis is still poorly understood (Arné *et al.*, 2011),

Chukar partridges are susceptible to several avian diseases, including pathogenic bacterial, viral and fungal agents, external and internal parasites, digestive disturbances, malnutrition, and inherited abnormalities (Del Hoyo *et al.*, 1994). However, most studies about partridge diseases are related to case reports of some wild or domestic populations (Haesendonck *et al.*, 2015; Belleau & Leonard, 1994; Vasilev, 1992; Rizzoli *et al.*, 1999). Only a little parasitological data is available on partridges in Turkey (Koroglu & Tasan, 1995; Koroglu & Tasan, 1996).

The sex ratio at birth is near to 1:1 in wild populations, but adult sex ratios are highly variable, suggesting that sex differences in post-birth maturation, mortalities, or population movements drive skewed adult sex ratios in birds. Important causes of mortality are pathogens or infectious agents (Székely *et al.*, 2014; Ancona *et al.*, 2017; Valdebenito at al., 2020).

The chukar partridge is one of the Turkey's most loved bird species due to its cultural and emotional bonds with Turkish people. Because of their singing behavior, they are traditionally fed at home, especially in villages. In addition, chukar partridges are one of the most important game birds. For these reasons, the Ministry of Agriculture and Forestry of Turkey has five chukar partridge breeding stations. The ministry produces partridges and releases them in suitable areas for hunting and restocking of the species. Chukar partridge diseases and the occurrence of contagious diseases are not well known. This study aims to evaluate the health condition of chukar partridges from four breeding stations and fourteen wild populations throughout Turkey by gross, histopathological method and the presence of Adenovirus, MD, AMB, and Aspergillosis using the immunohistochemical examination.

MATERIAL AND METHODS

Chukar sampling

Tissue samples were collected from wild and captive individuals during the 2018-2019 hunting seasons

in 13 cities (Bayburt (BAY), Bitlis (BIT), Burdur (BUR), Çanakkale (CAN), Çankiri, (CNR), Erzurum (ERZ), Eskisehir (ESK), Kahramanmaras (KAH), Kars (KAR), Konya (KON), Mugla (MUG), Sivas (SIV), Van (VAN)) and four breeding stations (Afyon (BSA), Kahramanmaras (BSK), Malatya (BSM), and Gaziantep (BSG)) throughout Turkey (Table 1). Captive adult individuals were selected from sick and injured birds in each breeding station for pathological examinations. The project was approved by the Local Ethical Committee on Animal Experiments of the Burdur Mehmet Akif Ersoy University, Turkey (Approval number: MAKU-HADYEK 169).

Molecular sexing method

Total DNA was extracted from blood or muscle tissues using GeneJET Whole Blood gDNA Purification Kit (Thermo Scientific). Molecular sex determination was performed using 2550 F (5'-GTTACTGATTCGTCTACGAGA-3') and 2718 R (5'-ATTGAAATGATCCAGTGCTTG-3') primers. These primers were designed to amplify homologous parts of CHD-W and the related gene CHD- Z. PCR conditions were as follows: 100 to 200 ng/µl genomic DNA, 0.5 µl (1 unit) for each primer (2550F, 2718R), 0.2 µl (1 unit) Taq DNA Polimerase (Fermentas), 0.2 µl (1 unit) dNTP mix (Fermentas), 1 µl MgCl2 (Fermentas), 2.5 µl 10X CL PCR Buffer (Invitrogen), 5 µl Q Solution (Invitrogen) and sterile dH2O up to a total volume of 25 µl. The PCR profile was performed with an initial denaturation step at 94°C for 7 min, followed by 30 cycles of 94°C denaturation for 60 s, annealing at 55°C for 120 s, and extension at 72°C for 60 s. A final 10 min extension at 72°C completed the PCR profile. PCR products were separated by electrophoresis for 60 min at 80 V in a 3% agarose gel stained with cyber green and visualized under UV light. Due to size differences between the W and Z fragments, females display two bands (W and Z copies) while males display one band (two copies of the Z fragment).

Histopathological Analysis

All visceral organ samples were collected from 224 partridges for pathological examination (Table 1). In addition, lesioned tissues were also harvested. Chukar partridge samples from wild and breeding stations were first examined macroscopically and then fixed in 10% neutral formalin. Recently dead birds with disease symptoms or traumatic lesions, or individuals that exhibited clinical signs at the breeding stations were selected for pathological examinations. Living birds were euthanatized by decapitation. All fixed samples were routinely processed using an automatic tissue processing equipment (Leica ASP300S, Leica Microsystem, Wetzlar, Germany), and embedded with paraffin wax and cut to approximately 5 µm with a Leica RM2155 rotary microtome (Leica Microsystems, Wetzlar, Germany). Slides were stained with hematoxylin and eosin (HE) for routine histopathological examination and examined under a light microscope.

Immunohistochemical examination

The streptavidin-biotin peroxidase complex method was applied to 4 different series sections on polylysine coated slides for the immunoperoxidase method. The Mouse and Rabbit Specific HRP/DAB Detection Kit –Micropolymer (ab236466) (Abcam Cambridge, UK) was used as the secondary antibody, and 3,3´-diaminobenzidine (DAB) was used as the chromogen. Tissue samples were immunostained with Adenovirus [Adenovirus Antibody (M58+M73) NB110-59908], Marek's disease [Marek Disease virus Antibody (14C8) NB120-3648], *Mycobacterium tuberculosis* [*Mycobacterium tuberculosis* Antibody NB100-65657], and Aspergillus [Aspergillosis Antibody (343/31) NB110-64511] according to the manufacturer's instructions. All primary antibodies were purchased from Novus Biologicals (CO, USA) and were used at a 1/100 dilution. For negative controls, antibody dilution solutions were used instead of primary antibodies. The Database Manual CellSens Life Science Imaging Software System (Olympus Corporation) was used for microphotography.

RESULTS

Wild and captive population samples were evaluated separately. 15.2% of 158 wild and 42.4% of 66 captive Chukar partridges had at least one disease or organ lesion. While 8.9% of the wild population had one organ lesion, 5.7% had two organ lesions, and 0.4% had three organ lesions, in the captive populations these figures were 24.2%, 9.1%, and 7.6%, respectively, and 1.5% of captive populations had four lesions (Table 1). The most frequent lesioned organs were livers (10.3%), lungs (8.5%), gastrointestinal system organs (5.8%), kidneys (2.7%), and pancreas (1.3%), respectively (Table 1). While wild males had a higher rate of the number of the lesions (14.9%), captive females had a lower rate (8.2%). Skin lesions were observed in 12 birds from breeding stations. We found kidney disease and Amyloidosis in only female chukars, and pancreas disease, Marek's disease, and Tuberculosis in only male chukars (Table 1).

Table 1 – Sample size in terms of location, histopathological lesion, disease location, and disease rates. Values are given as a percentage.

			n. of lesion				Disease location										
Area	n	healty	one	two	three	four	Liver	Lungs	Gastrointestinal	Kidney	Pancreas	Mycoplasma	Amyloidosis	Marek's disease	Aspergillosis	Adenovirus	Mycobacteriosis
Wild																	
BAY	4	100															
BIT	12	91.6		8.3			8.3								8.3		
BUR	19	94.7		5.3			5.3			5.3							
CAN	9	77.8	22.2				11.1	11.1									
CNR	16	81.3	18.8				18.8										
ERZ	22	72.7	13.6	9.1	4.5		18.2	9.1						13.6	4.5		
ESK	22	81.8	13.6	4.5			13.6		4.5		4.5						
KAH	4	75.0	25.0														25.0
KAR	3	100															
KON	3	100															
MUG	14	71.4	14.3	14.3			7.1	7.1	28.6								
SIV	12	91.7		8.3			8.3	8.3									
VAN	12	100															
6 cities	6	83.0	17.0														17.0
Captive																	
BSA	13	46.2	38.5	7.7	7.7		15.4	7.7	30.8	7.7			15.4				
BSK	31	48.4	22.6	12.9	12.9	3.2	16.1	41.9	12.9	6.5	3.2	3.2	3.2			12.9	
BSM	19	84.2	15.8			,	5.3				5.3				5.3		
BSG	3	33.3	33.3	33.3						66.6			33.3				
Total																	
Wild	158	84.8	8.9	5.7	0.4		9.5	3.2	3.2	0.6	0.6			1.9	1.3		1.3
BS	66	57.6	24.2	9.1	7.6	1.5	12.1	21.2	12.1	7.6	3.0	1.5	6.1		1.5	6.1	
Grand	224	76.8	13.4	6.5	2.7	0.4	10.3	8.5	5.8	2.7	1.3	0.4	1.8	1.3	1.3	1.8	0.9
Gender																	
Wild																	
Female	49	91.8	4.1	4.1			6.1	4.1		2.0							
Male	54	85.1	9.3	3.7	1.9		11.1	3.7			1.9			1.9	1.9		1.9
Captive																	
Female	17	47.2	29.4	5.8	17.6		23.5	11.8	29.4	5.9			5.9		5.9	11.8	
Male	17	58.8	23.5	11.8	5.9		11.8	29.4	17.6							5.9	
Total																	
Female	66	80.3	10.7	4.5	4.5		10.6	6.1	7.6	3.0			1.5		1.5	3.0	
Male	71	78.9	12.7	5.6	2.8		11.3	9.9	4.2		1.4			1.4	1.4	1.4	1.4

Clinical and Necropsy Findings

Birds with evidence of disease or disability were evaluated, and the clinical findings of these partridges were noted. Clinically, traumatic lesions and inflammatory reactions were mostly observed in the eyes (12 out of 66, 18.18%) and extremities (10 out of 66, 15.15%) of the Chukar partridges in the breeding stations. During the necropsy, the gross lesions in the organs were examined and recorded. The clinical findings could not be evaluated because the samples from the hunters were presented fixed in formaldehyde, but the presence of macroscopic lesions of the organs was examined before processing the tissues. Macroscopic findings of animals in breeding stations were evaluated. Traumatic lesions and inflammations were commonly observed in the eyes and extremities of the partridges in the breeding station. The most common findings in the sick partridges were lethargy and fluffed-up plumage closed eyes and sitting position, respectively. Some birds exhibited nervous symptoms. Caseous masses were noticed under the skin in three partridges (Fig.1). In addition, traumatic lesions such as feather loss and leg fractures or skin ulcers were frequently observed. Foot problems and toe anomalies were among the other common findings (Fig.2).

Figure 1 – Gross lesions of chukar partridges: (A) subcutaneous caseous necrosis formation, (B) corneal ulcer, (C) periorbital inflammation and swelling, (D) opisthotonos.

At necropsy, the most common finding was enteritis and liver lesions found in respectively 23 [14 captive (9 males and 5 females) and 9 wild (6 males and 3 females)] and 16 out [10 captive (6 males and 4 females) and 6 wild (4 males and 2 females)] of 224 birds (respectively 10.26% and 7.14%). Among the findings encountered in the necropsies of the 224 chukar partridges were granulomatous lesions in the abdominal cavity (0.4%, 1 case), splenomegaly (3.6%, 8 cases), necrosis (0.8%, 2 cases), color changes in the livers (2.2%, 5 cases), and intestinal hemorrhages (2.2%, 5 cases) (Fig. 3).

Figure 2 – Leg problems of chukar partridges: A: leg paralysis, B: decubitus, and C-D: arthritis.

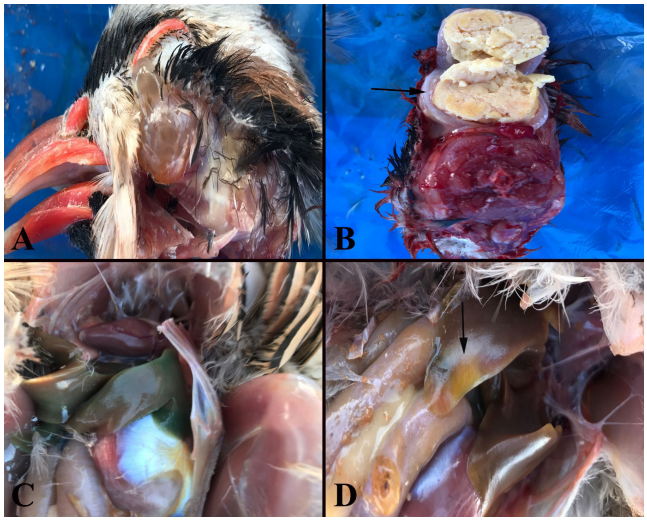

Figure 3 – Necropsy findings of chukar partridges: A: periorbital swelling, B: subcutaneous caseous necrosis formation (arrow), C: hepatic discoloration, D: lipidosis (arrow) in the liver.

Histopathological Findings

At the histopathological examination, most of the visceral organs of the birds had normal appearance (84.8% in the wild population; 57.6% in the captive population). However, some pathological lesions were observed in both birds collected from breeding stations and natural environments. It was noted that the lesions were generally localized in the lungs, livers, and intestines (Table 1). Generally inflammatory reaction and necrotic areas were observed in the organs. Anthracosis indicates air pollution and is commonly observed in the lungs, especially in partridges from BSK (38.7%, 12 cases). Moreover, liver parasites are commonly observed in birds from the wild population (6.3%, 10 cases; Fig. 4).

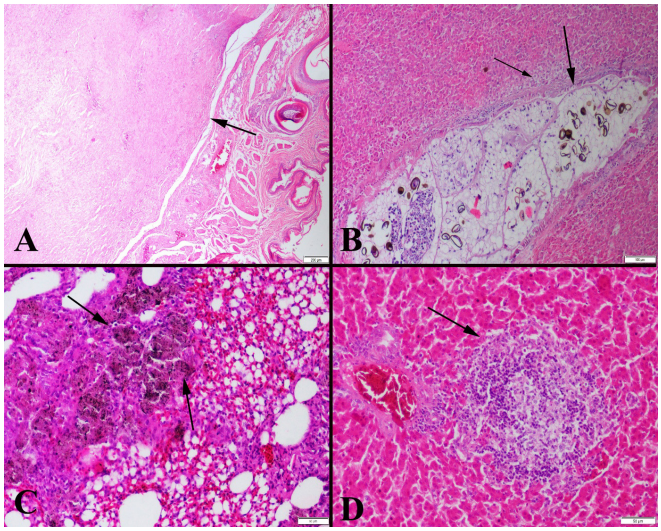

Figure 4 – Representative histopathological findings: A: subcutaneous necrotic mass (arrow), B: liver parasite (arrow), C: severe anthracosis (arrows), D: inflammatory cell infiltrations (arrow), HE, scale bars=50µm.

Immunohistochemistry results

MD virus antigens were found in wild ERZ population (13.6%, 3 cases), Aspergillosis in BIT and ERZ wild populations (8.3% and 4.5%, 2 cases) and in BSM (5.3%, 1 case). Adenovirus were found in 3 cases from the breeding station, and AMB was also diagnosed in 4 chukar partridges from wild populations (2 cases in KAH and 2 cases in ERZ) (Table 1). While Adenovirus positive immunoreaction was found in the lungs, MD antigens have been detected in the lungs and spleens. Aspergillus antigens have also been found in the lungs. Mycobacteriosis antigen-positive reaction was observed in the lungs and a granuloma was located in the abdominal cavity (Fig. 5).

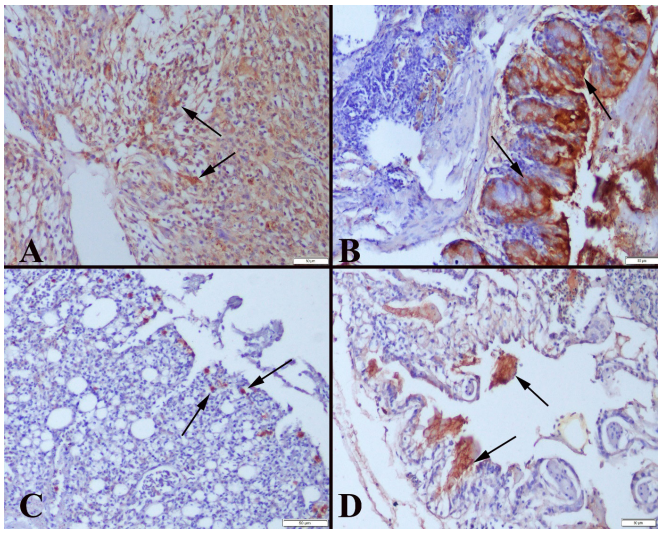

Figure 5 – Immunohistochemical findings of chukar partridges: A: Mycobacteriosis positive reaction in inflammatory cells (arrows) in a granuloma, B: Adenovirus positive immunoreaction (arrows) in a bronchiolar epithelial cell, C: MD antigen-positive immunoreaction (arrows) in alveolar epithelial cells, D: Aspergillus positive reaction in alveolar macrophages (arrows) in a lung, Streptavidin biotin peroxidase method, scale bars = 50µm.

MD and AMB, which cause high mortality in birds, were not diagnosed in breeding stations. These diseases were only diagnosed in wild populations (Table 1). Aspergillosis and Adenovirus antigens were detected in captive populations, respectively BSM and BSK. However, since these are not related to any epidemic, they were considered individual cases. Aspergillosis was found in BIT (8.3%, 1 case in BIT population) and ERZ (4.5%, 1 case in ERZ population), in wild populations. The detailed distribution of the examined diseases in Turkey is shown in Fig. 6 and Table 1.

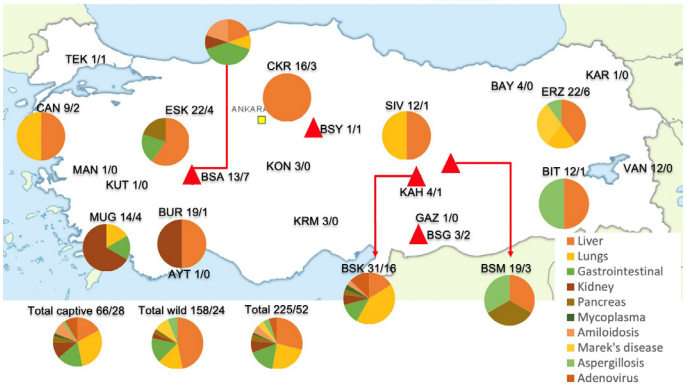

Figure 6 – Diseases and lesions detected in partridge and their distribution by province. The number of samples and the number of patients is given in the sample areas where pie charts are not provided.

Only meager disease rates were detected in the samples collected from the wild and breeding station populations, and pathological examinations found no significant contamination from the populations of both captive and wild Chukar partridges. A total of 224 birds were examined, and an incidence of MD 1.3%, AMB 0.9%, Aspergillosis 1.3% and Adenovirus infection 1.8% were found. Moreover, numerous liver parasites were observed in the histological sections, but they were not identified. Additionally, lung, liver, kidney, and gastrointestinal system lesions were detected in both captive and wild populations (Table 1).

Molecular sexing results

In terms of sex and diseases relation, we found kidney lesions and Amyloidosis in only female chukars, and pancreas lesions, Marek's disease, and mycobacteriosis only in male chukars. Liver, lung, gastrointestinal system lesions, aspergillosis, and adenovirus infections were observed in both sexes (Fig. 7).

DISCUSSION

Chukar partridge is a popular game bird enjoyed by many Turkish people like the red-legged partridge (*Alectoris rufa*) in many parts of Europe (Millan, 2009). The chukar partridge is popular game bird in Middle

Figure 7 – Molecular analysis results of sex determinations, one band is male, and two bands is female.

Asia (Mahmood *et al.*, 2019). There is little knowledge about chukar partridge diseases, not only for wild populations but also for breeding stations in Turkey. Adenovirus, MD, AMB, and Aspergillosis infections are important and contagious diseases for birds. These diseases were found in both captive and wild populations (Morrow & Fehler, 2004; Koroglu & Tasan, 1995; Guillot *et al.*, 2001; McConnell & Fitzgerald, 2001), but there is no pathological description in previous studies.

Fowl Adenoviruses can cause important and fatal diseases in both domestic and wild birds depending on the strain virulence, but they can also be isolated in healthy birds throughout the world (McConnell & Fitzgerald, 2001). Adenoviral outbreaks have been reported in more than 40 wild-bird species by many authors (Deuchande *et al.*, 2012). We found a total of 3 cases of Adenovirus positive reaction were diagnosed in chukar partridges, 2 of them from breeding stations and one from the wild population. However, no characteristic necropsy or histopathology findings were observed in any of the birds.

MD one of the most common infectious causes of poultry mortality (Deuchande *et al.*, 2012). Chukar partridges produced in five breeding stations in Turkey looks like poultry from the industry. Vaccination against the MD has substantially reduced losses in the commercial poultry industry. However, the disease may be diagnosed in unvaccinated birds and cause high mortality. Apart from chickens, the disease has been described rarely in wild birds such as crested partridges (*Rollulus rouloul*), in which ocular lesions resulting in blindness were reported (Haesendonck *et al.*, 2015). Because the vaccinations were applied in all breeding stations, no MD cases were diagnosed in captive populations in this study. In addition, none of the chukar partridges from the wild populations exhibited any macroscopic findings related the MD. In this study, no histopathological lesions related to the diseases were observed either. Nevertheless, MD antigens were detected in 4 partridge tissues collected from the wild populations.

AMB is an important chronic infectious disease that affects domestic and wild birds worldwide, as well as exotic species (Fulton & Thoen, 2003). The disease may be present in a variety of clinical forms, with most organ systems being affected. AMB has been reported as the cause of death in 1 to 30% of raptor cases examined post mortem (Millan, 2009). Generally, the primary source of the disease is infected birds, contaminated water, food or soil. Agents can survive for several months under suitable environmental conditions (Fulton & Thoen, 2003). Inappetence, diarrhea, fluffy and opaque feathers, loss of weight, and mortality are commonly observed clinical symptoms (Converse, 2007; Muttalib & Riddell, 1988). In characteristic and chronic cases, multiple granulomas with a yellowish to whitish caseonecrotic center commonly occur in visceral organs and the skin (Converse, 2007; Muttalib & Riddell, 1988). Although none of the 224 examined chukar partridges exhibited any clinical symptoms or necropsy findings related to mycobacteriosis, agents were detected in four birds from the field through the immunohistopathological method in this study.

The incidence and importance of AMB in captive collections of other birds are increasing (Painter, 1997). AMB affects avian species, especially waterfowl, Galliformes, Columbiformes, Passeriformes, Psittaciformes, and Accipitriformes (Dhama, *et al.* 2008). However, this disease is not well known in wild or captive birds (Marco *et al.*, 2000; Millan *et al.*, 2010). Susceptibility to disease and occurrence of the symptoms varies from species to species. Bird species may be classified into four groups according to their susceptibility to disease as highly susceptible, less susceptible, moderately resistant, and highly resistant. The partridges are in the group of most susceptible birds (Hejlicek & Treml, 1993), and stress factors appear to increase the incidence and severity of the disease in captive birds (Aranaz et.al., 1997) Therefore, the breeding stations ought to monitor for these kinds of diseases and reduce the stress factors in productive strategies. The main reason for negative incidence may be related to the restrictive health control in the breeding stations. The lower incidence in wild populations may be attributed to natural selection.

Aspergillosis is called "brooder pneumonia", and the most common clinical symptoms are dyspnea,

anorexia, diarrhea, and sudden death. Gross lesions are most frequently found at necropsy in the respiratory system, especially lungs and air sacks (Ozmen & Dorrestein, 2004; Tell, 2005; Martin, 2007). *Aspergillus fumigatus* is the most commonly isolated agent, but *Aspergillus flavus* or *Aspergillus niger* may cause the disease (Martin, 2007). Although we did not find any Aspergillosis in wild populations, 3 cases were diagnosed from the breeding stations. These findings agree with the previous reports about diseases related to environmental conditions, and crowded flocks were predisposing factors (Ozmen & Dorrestein, 2004; Tell, 2005; Martin, 2007).

There is little knowledge about endoparasites in bird livers, especially chukar partridges. A few digenetic trematodes, such as *Brachylaemus fuscatus*, *Dicrocoelium petrovi,* and *Dicrocoelium* sp., have been reported from partridges from Europe (Haesendonck *et al.*, 2015; Belleau and Leonard, 1994; Vasilev, 1992; Rizzoli *et al.*, 1999; Calvete *et al.*, 2003). There is no report about liver fluke in chukar partridges in Turkey. Although we observed numerous liver trematoda in the histological sections, they were not identified.

Immunohistochemically, fatal diseases such as MD and AMB were not observed at breeding stations. Adenovirus and Aspergillosis cases have been encountered very rarely. Humid environments may have caused Aspergillosis in breeding stations. For this reason, it is thought that these problems will disappear when attention is paid to hygiene.

The high rate of hepatitis in the breeding stations (6 of 12 cases in the breeding stations) suggested that attention should be paid to feeding and feed quality. In addition, the high rate of pneumonia and anthracosis cases detected in the KHR breeding station showed that there was a problem with air quality in this region. The high eye problems in the KHR breeding station made us think it was caused by crowded flocks. However, it was thought that the samples taken from breeding stations were collected only when showing disease symptoms, which contributed to this rate.

These study findings showed that birds do not exhibit an overall sexual difference in disease prevalence and mortality. However, the limitations in our analysis and the small number birds included the study may have contributed to this lack of association.

CONCLUSION

As a result, this study shows that chukar partridges in both breeding stations and wild populations are not an important source of disease, but chukar partridges released from breeding stations might carry some microorganisms. However, it is thought that they may be effective in transmitting agents to the other wild animals that connect with them in the same environment, especially its predators. This study results also indicated that partridges are not a threat to human health.

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